

Department of Obstetrics and Gynecology
Helsinki University Central Hospital
University of Helsinki, Finland

PLACENTAL ABRUPTION

Studies on incidence, risk factors and potential predictive biomarkers

Minna Tikkanen

Academic Dissertation

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Supervised by

Professor Jorma Paavonen, M.D., Ph.D.
Department of Obstetrics and Gynecology
University of Helsinki

and

Professor Olavi Ylikorkala, M.D., Ph.D.
Department of Obstetrics and Gynecology
University of Helsinki

Reviewed by

Docent Eeva Ekholm, M.D., Ph.D.
Department of Obstetrics and Gynecology
University of Turku

and

Docent Jukka Uotila, M.D., Ph.D.
Department of Obstetrics and Gynecology
University of Tampere

Official opponent

Professor Seppo Saarikoski, M.D., Ph.D.
Department of Obstetrics and Gynecology
University of Kuopio

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred by their Roman numerals in the text:

- I** Tikkanen M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Prepregnancy risk factors for placental abruption. *Acta Obstet Gynecol Scand* 2006; 85: 40-44.
- II** Tikkanen M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Clinical presentation and risk factors for placental abruption. *Acta Obstet Gynecol Scand* 2006; 85: 700-705.
- III** Tikkanen M, Hämäläinen E, Nuutila M, Paavonen J, Ylikorkala O, Hiilesmaa V. Elevated maternal second-trimester serum alpha-fetoprotein as a risk factor for placental abruption. *Prenat Diagn* 2007; 27: 240-243.
- IV** Tikkanen M, Stenman U-H, Nuutila M, Paavonen J, Hiilesmaa V, Ylikorkala O. Failure of second trimester measurement of soluble endoglin and other angiogenic factors to predict placental abruption. *Prenat Diagn* 2007; 27: 1143-1146.
- V** Tikkanen M, Surcel H-M, Bloigu A, Nuutila M, Hiilesmaa V, Ylikorkala O, Paavonen J. Prediction of placental abruption by testing for C-reactive protein and chlamydial antibody levels in early pregnancy. *BJOG* 2008; 115: 486-491.

ABBREVIATIONS

BMI	body mass index
CHSP60	chlamydial heat shock protein 60
CI	confidence interval
CRP	C-reactive protein
C/S	cesarean section
CTG	cardiotocography
DIC	disseminated intravascular coagulopathy
dwk	decimal weeks
ELISA	enzyme linked immunosorbent assays
HLA	human leucocyte antigens
IL	interleukin
IQR	interquartile range
IUGR	intrauterine growth restriction/retardation
MMP	matrix metalloproteinase
MoM	multiples of median
MSAFP	maternal serum alpha-fetoprotein
MSβ-hCG	maternal serum free beta human chorionic gonadotrophin
MTHFR	methylenetetrahydrofolate reductase
NK cells	natural killer cells
NS	not significant
OR	odds ratio
PIH	pregnancy induced hypertension
PlGF	placental growth factor
PMR	perinatal mortality rate
PROM	premature rupture of the membranes
ROC	receiver operating characteristic
RR	relative risk
SD	standard deviation
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase 1
SGA	small for gestational age
TNF-α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor

ABSTRACT

Placental abruption, one of the most significant causes of perinatal mortality and maternal morbidity, occurs in 0.5-1% of pregnancies. Its etiology is unknown, but defective trophoblastic invasion of the spiral arteries and consequent poor vascularization may play a role. The aim of this study was to define the prepregnancy risk factors of placental abruption, to define the risk factors during the index pregnancy, and to describe the clinical presentation of placental abruption. We also wanted to find a biochemical marker for predicting placental abruption early in pregnancy.

Among women delivering at the University Hospital of Helsinki in 1997-2001 (n=46,742), 198 women with placental abruption and 396 control women were identified. The overall incidence of placental abruption was 0.42%. The prepregnancy risk factors were smoking (OR 1.7; 95% CI 1.1, 2.7), uterine malformation (OR 8.1; 1.7, 40), previous cesarean section (OR 1.7; 1.1, 2.8), and history of placental abruption (OR 4.5; 1.1, 18). The risk factors during the index pregnancy were maternal (adjusted OR 1.8; 95% CI 1.1, 2.9) and paternal smoking (2.2; 1.3, 3.6), use of alcohol (2.2; 1.1, 4.4), placenta previa (5.7; 1.4, 23.1), preeclampsia (2.7; 1.3, 5.6) and chorioamnionitis (3.3; 1.0, 10.0). Vaginal bleeding (70%), abdominal pain (51%), bloody amniotic fluid (50%) and fetal heart rate abnormalities (69%) were the most common clinical manifestations of placental abruption. Retroplacental blood clot was seen by ultrasound in 15% of the cases. Neither bleeding nor pain was present in 19% of the cases. Overall, 59% went into preterm labor (OR 12.9; 95% CI 8.3, 19.8), and 91% were delivered by cesarean section (34.7; 20.0, 60.1). Of the newborns, 25% were growth restricted. The perinatal mortality rate was 9.2% (OR 10.1; 95% CI 3.4, 30.1).

We then tested selected biochemical markers for prediction of placental abruption. The median of the maternal serum alpha-fetoprotein (MSAFP) multiples of median (MoM) (1.21) was significantly higher in the abruption group (n=57) than in the control group (n=108) (1.07) (p=0.004) at 15-16 gestational weeks. In multivariate analysis, elevated MSAFP remained as an independent risk factor for placental abruption, adjusting for parity ≥ 3 , smoking, previous placental abruption, preeclampsia, bleeding in II or III trimester, and placenta previa. MSAFP ≥ 1.5 MoM had a sensitivity of 29% and a false positive rate of 10%. The levels of the maternal serum free beta human chorionic gonadotrophin MoM did not differ between the cases and the controls. None of the angiogenic factors (soluble endoglin, soluble fms-like tyrosine kinase 1, or placental growth factor) showed any difference between the cases (n=42) and the controls (n=50).

in the second trimester. The levels of C-reactive protein (CRP) showed no difference between the cases (n=181) and the controls (n=261) (median 2.35 mg/l [interquartile range {IQR} 1.09-5.93] versus 2.28 mg/l [IQR 0.92-5.01], not significant) when tested in the first trimester (mean 10.4 gestational weeks). *Chlamydia pneumoniae* specific immunoglobulin G (IgG) and immunoglobulin A (IgA) as well as *C. trachomatis* specific IgG, IgA and chlamydial heat-shock protein 60 antibody rates were similar between the groups.

In conclusion, although univariate analysis identified many prepregnancy risk factors for placental abruption, only smoking, uterine malformation, previous cesarean section and history of placental abruption remained significant by multivariate analysis. During the index pregnancy maternal alcohol consumption and smoking and smoking by the partner turned out to be the major independent risk factors for placental abruption. Smoking by both partners multiplied the risk. The liberal use of ultrasound examination contributed little to the management of women with placental abruption.

Although second-trimester MSAFP levels were higher in women with subsequent placental abruption, clinical usefulness of this test is limited due to low sensitivity and high false positive rate. Similarly, angiogenic factors in early second trimester, or CRP levels, or chlamydial antibodies in the first trimester failed to predict placental abruption.

INTRODUCTION

Placental abruption, defined as the complete or partial separation of the placenta before delivery, is one of the leading causes of vaginal bleeding in the second half of pregnancy (Konje and Taylor. 2001, Oyelese and Ananth. 2006). Approximately 0.5-1% of the pregnancies are complicated by placental abruption (Kyrklund-Blomberg et al. 2001, Oyelese and Ananth. 2006). Bleeding and pain are classical symptoms of abruption but the clinical picture of this emergency varies (Konje and Taylor. 2001, Oyelese and Ananth. 2006). Placental abruption is one of the most important causes of maternal morbidity and perinatal mortality. Approximately 10% of all preterm births and up to one third of all perinatal deaths are caused by placental abruption (Ananth et al. 2006a, Oyelese and Ananth. 2006). In many countries the rate of placental abruption has been increasing (Saftlas et al. 1991, Ananth and Wilcox. 2001), perhaps due to advancing maternal age and increasing cesarean section rates (Saftlas et al. 1991, Rasmussen et al. 1996, Ananth et al. 2005).

Although several risk factors are known, the cause of placental abruption often remains unexplained. The trophoblastic invasion in the spiral arteries and subsequent early vascularisation may be defective (Dommissse and Tiltman. 1992, Kraus et al. 2004). Moreover, placental abruption may also be a manifestation of an inflammatory process which could affect also vascular bed (Ananth et al. 2006b). Despite heightened awareness of placental abruption, it still remains largely unpredictable and therefore also unpreventable. A reliable biochemical marker to detect individuals at risk before clinical emergency would be most useful in clinical practice. Although several markers have been studied (Nolan et al. 1993, Bartha et al. 1997, Chandra et al. 2003, Florio et al. 2003, Dugoff et al. 2005, Signore et al. 2006), none has so far emerged as clinically useful.

The present studies were designed to more definitively define prepregnancy risk factors for placental abruption, to study risk factors of placental abruption during the index pregnancy, and to describe the clinical presentation of placental abruption. We also wanted to find a new biochemical marker in order to predict placental abruption in early pregnancy.

REVIEW OF THE LITERATURE

General aspects

The placenta is a unique organ providing oxygen, nourishment, and protection to the fetus and having excretory and endocrine functions. After repeated mitotic divisions the zygote transforms into a blastocyst. The blastomeres of the blastocyst form an outer shell of cells, called trophoblast and a localized, inner cell mass, the embryoblast. After attaching to the endometrium the trophoblast cells rapidly proliferate and differentiate into an outer layer of syncytiotrophoblast and an inner layer of cytotrophoblasts (Faye-Petersen et al. 2006). The syncytiotrophoblasts form primary, secondary and finally tertiary villi and cytotrophoblasts form intervillous space. The placenta is fixed to the uterine wall by anchoring villi. By the end of the fourth month of gestation, the placenta has achieved its definitive form and undergoes no further anatomic modification. Growth, branching of the villous tree, and formation of fresh villi continues until term (Fox. 1999).

Implanted placenta naturally separates during the third part of the labor. The separation process is multiphasic: latent (placental site wall remains thin while placenta-free wall is thick), contraction (thickening of placental site wall), detachment (actual separation of the placenta from the adjacent uterine wall), and expulsion (sliding of the placenta out of the uterine cavity). Uterine contractions cause the separation of the placenta (Herman et al. 2002).

Definition

Placental abruption is classically defined as complete or partial premature separation of a normally implanted placenta with hemorrhage into the decidua basalis (Konje and Taylor. 2001, Oyelese and Ananth. 2006). Antepartum hemorrhage, i.e. bleeding after the 20th week of pregnancy occurs in 2-5% of all pregnancies and placental abruption accounts for approximately one quarter of such cases (Konje and Taylor. 2001). The diagnosis of placental abruption is always clinical (Faye-Petersen et al. 2006, Oyelese and Ananth. 2006) and the condition should be suspected in women who present with vaginal bleeding or abdominal pain or both, a history of trauma, and in those who present with otherwise unexplained preterm birth (Oyelese and Ananth. 2006). Symptoms of abruption vary immensely from an asymptomatic form in which the diagnosis is made only on placental inspection at delivery to massive abruption leading to fetal death and severe maternal morbidity (Oyelese and Ananth. 2006). The rate of the abruption

is notably higher (4%) when the diagnosis is made by a pathologist and most of these cases have an unremarkable obstetric history (Faye-Petersen et al. 2006).

The clinical classification of placental abruption is based on the observation of bleeding at three principal sites (Figure 1). The bleeding can be subchorionic (between the myometrium and the placental membranes), retroplacental (between the myometrium and the placenta), or preplacental (between the placenta and amniotic fluid) (Nyberg et al. 1987). Subchorionic hematomas may be remote from the placenta but are thought to rise from marginal abruptions. Preplacental hemorrhage includes both subamniotic hematoma and massive subchorionic thrombosis (Nyberg et al. 1987, Oyelese and Ananth. 2006). Intraplacental hematoma also occurs (Kraus et al. 2004). Abruption may be “revealed”, in which cases blood tracks between the membranes and the decidua escaping through the cervix into the vagina (Oyelese and Ananth. 2006). This occurs in 65-80% of cases (Konje and Taylor. 2001). The less common “concealed” abruption occurs when blood accumulates behind the placenta, with no obvious external bleeding (Oyelese and Ananth. 2006). This happens in 20-35% of cases (Konje and Taylor. 2001), Figure 2. The concealed type is most dangerous with more severe complications (Konje and Taylor. 2001). Finally, abruption may be total, involving the entire placenta, in which case it typically leads to fetal death, or partial with only a portion of the placenta detached from the uterine wall (Oyelese and Ananth. 2006). Partial abruption is more common.

Figure 1.

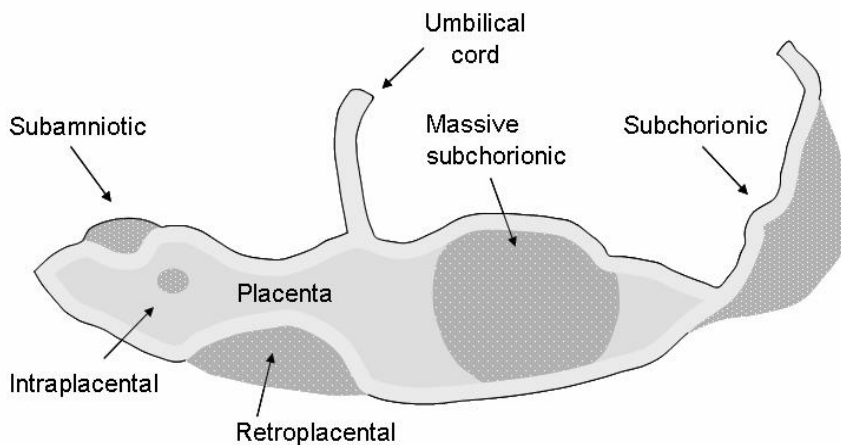
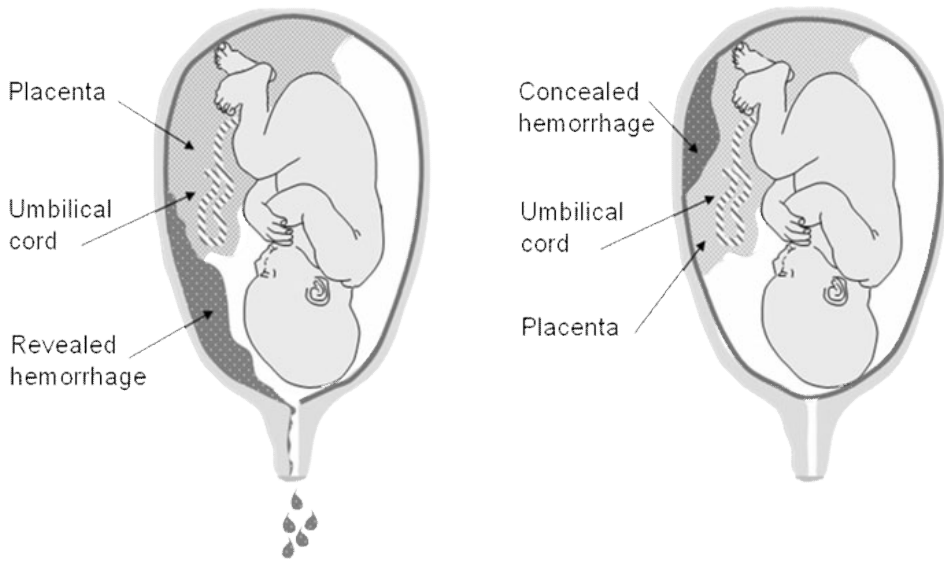


Figure 2.



Epidemiology

The overall incidence of placental abruption varies from 0.5 to 1.0% (Ananth et al. 1996, Ananth et al. 1999a, Baumann et al. 2000, Ananth and Wilcox. 2001, Kyrklund-Blomberg et al. 2001). The rate is lower in case-control (0.35%) than in cohort studies (0.69%) (Ananth et al. 1999a). In the United States-based studies the incidence has been higher both in cohort (0.81%) and case-control (0.37%) studies compared with studies conducted outside the U.S. (0.60% and 0.26%, respectively) (Ananth et al. 1999a). The incidence may vary due to variable diagnostic criteria (Konje and Taylor. 2001). The incidence is highest at 24-26 weeks of gestation, and drops with advancing gestation (Rasmussen et al. 1996, Oyelese and Ananth. 2006). However, it occurs after the 36th week of gestation in about 50% of cases (Konje and Taylor. 2001). Some (Saftlas et al. 1991, Ananth et al. 2005a, Rasmussen et al. 1996) but not all (Ananth and Chattingius. 2007) studies have reported increasing overall rates.

Abruptio occurs more frequently in older women, but usually this increase has been attributed to multiparity (≥ 3 deliveries), and is independent of age (Baumann et al. 2000, Konje and Taylor. 2001). However, the literature provides conflicting evidence with respect to whether age and parity are associated with placental abruption (Kåregård and Gennser. 1986, Krohn et al. 1987, Rasmussen et al. 1996, Kramer et al. 1997, Baumann et al. 2000, Lindqvist and Happach.

2006). In one study neither parity nor maternal age increased the risk (Krohn et al. 1987). In another study maternal age > 35 years predicted placental abruption among primiparous but not among multiparous women (Baumann et al. 2000). In many other studies advanced maternal age has been an independent risk factor (Rasmussen et al. 1996, Kramer et al. 1997, Lindqvist and Happach. 2006,). Also mothers less than 20 years of age have been a risk group in some studies (Kåregård and Gennser. 1986, Saftlas et al. 1991). Being black, unmarried, or of lower socioeconomic status are other risk factors for abruption (Krohn et al. 1987, Saftlas et al. 1991, Kramer et al. 1997, Ananth et al. 1999b).

Maternal consequences

Maternal risks associated with placental abruption depend primarily on the severity of the abruption (Oyelese and Ananth. 2006). Peripartum risks include obstetric hemorrhage, need for blood transfusions, hysterectomy, disseminated intravascular coagulopathy (DIC), renal failure and less commonly, maternal death (Oyelese and Ananth. 2006). Placental abruption attributes to nearly a quarter of late pregnancy bleeding (Konje and Taylor. 2001). Bleeding can sometimes lead to maternal hypovolemic shock. Blood loss may be underestimated in placental abruption because concealed bleeding into the myometrium is difficult to quantify (Konje and Taylor. 2001). The coagulation cascade becomes activated with consumption of coagulation factors and platelets. Thrombin converts fibrinogen to fibrin, and the stable fibrin clot is the final product of hemostasis. The fibrinolytic system then breaks down fibrinogen and fibrin. In the presence of thrombin, activation of the fibrinolytic system generates plasmin, which is responsible for the lysis of fibrin clots. When the placental detachment is large enough to cause fetal death, the risk of DIC is high. In this condition, coagulation and fibrinolysis happen without control which results in simultaneous widespread clotting and bleeding. Placental abruption may also be associated with acute renal failure resulting from hypovolemia or DIC (Konje and Taylor. 2001). Maternal mortality decreased from 8% in 1919 to less than 1 % in 1995 (Konje and Taylor. 2001). In the United Kingdom in 2000-2002, four maternal deaths were caused by placental abruption (Konje and Taylor. 2001). Fetomaternal hemorrhage can lead to severe immunization in Rhesus-negative patients (Konje and Taylor. 2001).

Women who have had placental abruption are less likely than other women to become pregnant again (Rasmussen et al. 1997). After placental abruption with survived newborn 59% of women had subsequently another delivery, compared with 71% of those without abruption. After perinatal loss corresponding rates were 83% and 85%, respectively (Rasmussen et al. 1997). This may reflect maternal anxiety and distress caused by placental abruption.

Having placental abruption has further effects on subsequent maternal health. For instance, the risk of premature cardiovascular disease is increased by 70% in these women (Ray et al. 2005). The cause of this is unclear.

Perinatal consequences

Placental abruption is associated with low birth weight, preterm delivery, hypoxia, stillbirth and perinatal death (Ananth et al. 1999b). Fetal survival depends on the severity of the abruption and the gestational age (Oyelese and Ananth. 2006). Abruption involving more than 50% of placental surface is frequently associated with fetal death (Ananth et al. 1999b, Oyelese and Ananth. 2006). A population based cohort study showed perinatal mortality rate (PNM) of 11.9% among pregnancies complicated by abruption, compared with 0.8% in other births (Ananth and Wilcox. 2001). The high PNM with abruption can be explained by the strong association with preterm delivery. However, even term babies with normal birth weight have a 25-fold higher mortality with abruption (Ananth and Wilcox. 2001). Also, the PNM depends to some extent on neonatal facilities. Over 50% of the perinatal deaths are stillborns (Konje and Taylor. 2001).

Perinatal mortality is closely related to gestational age. Placental abruption may be implicated in up to 10% of all preterm births (Ananth et al. 1999b). Although placental abruption is an important cause of spontaneous preterm birth, it also causes iatrogenic preterm delivery (Ananth et al. 1999b). In this study the rate for preterm birth among women with placental abruption was 39.6% compared to 9.1% in women without (Ananth et al. 1999b). Approximately 18 % of the abruptions occur before 32 weeks and 42% occur after 37 weeks (Konje and Taylor. 2001). Prematurity poses serious threat to the fetus with short-term and long-term neonatal consequences (Ananth et al. 1999a).

Preterm birth is often associated with birth weight < 2500 g. In one study the rate of giving birth to a low-birth weight infant among women with placental abruption was 46% compared to 6.4% among those without (Ananth and Wilcox. 2001). Other consequences include fetal growth restriction, anemia, and hyperbilirubinemia of the newborn (Hladky et al. 2002). The association with fetal growth restriction is so strong that growth restriction alone could be used as a marker for the risk of abruption (Ananth and Wilcox. 2001). The rate of fetal malformations may be as high as 4.4% which is 2-times higher than that in general population. Most involve congenital heart defects and central nervous system (Raymond and Mills. 1993, Konje and Taylor. 2001). The cause for this is unclear.

Premature separation of placenta deprives the fetus of oxygen and nourishment (Oyelese and Ananth. 2006). In severe cases Apgar scores and cord blood pH values are often low due to antenatal hypoxia and blood loss (Spinillo et al. 1993, Toivonen et al. 2002, Matsuda et al. 2003, Allred and Batton. 2004). In one study the risk for intrapartum asphyxia with placental abruption was 3.7-fold. Three percent of asphyctic newborns and 0.7% of controls had placental abruption (Heinonen and Saarikoski. 2001). Intrapartum asphyxia may lead to long-term consequences among survivors. Neonates born after placental abruption are more likely to develop cystic periventricular leucomalasia or intraventricular hemorrhage (Spinillo et al. 1993, Gibbs and Weindling. 1994). The risk increases with prematurity and low birth weight (Spinillo et al. 1993, Gibbs and Weindling. 1994). Severe abruption increases the risk for cerebral palsy (Spinillo et al. 1993, Thorngren-Jerneck and Herbst. 2006). Placental abruption is also associated with sudden infant death syndrome (Klonoff-Cohen et al. 2002, Getahun et al. 2004).

Etiology

Placental abruption seems to be a multifactorial disease. Its etiology is not fully understood but impaired placentation, placental insufficiency, intrauterine hypoxia, and uteroplacental underperfusion are the key mechanisms causing abruption (Ananth et al. 1997, Kramer et al. 1997, Rasmussen et al. 1999, Ananth et al. 2006a). Abruption results from a rupture of maternal decidual artery causing a dissection of blood at the decidual-placental interface, around placental margin, or behind the membranes (Faye-Petersen et al. 2006). Acute vasospasm of small vessels may be one event immediately preceding placental separation. Thrombosis of the decidual vessels with associated decidual necrosis and venous hemorrhage also are often present (Oyelese and Ananth. 2006). In some cases, blunt trauma or rapid decompression of the overdistended uterus cause abruption but in most cases placental abruption seems to be a consequence of a long-standing process perhaps dating back to the first trimester (Ananth et al. 2006b).

Immunological rejection

Immunological defects may play a role in the origin of placental abruption (Matthiesen et al. 1995, Steinborn et al. 2003b). These defects may lead to an excessive maternal inflammatory response with increased release of cytokines and result in a chain of events including shallow trophoblast invasion, defective spiral artery remodeling, placental infarctions and thrombosis (Matthiesen et al. 2005). Excessive activation of the immune system may suggest past exposure

to major antigens (Steinborn et al. 2004). Cell-mediated immunity is suppressed and humoral immune response is upregulated in normal pregnancy but not in placental abruption (Matthiesen et al. 1995, Steinborn et al. 2004). This can then lead to exaggerated maternal immune rejection of the fetus, activation of fetal monocytes and release of inflammatory agents (Steinborn et al. 2004, Nielsen et al. 2007). Trophoblastic cells interact in the decidua with natural killer (NK) cells which express receptors that recognize combinations of human leukocyte antigens (HLA). HLA-G levels, decisive factors for the avoidance of rejection of the fetus, are strongly decreased in women with placental abruption (Steinborn et al. 2003a). High level of soluble HLA-G is needed to switch cytokine profile towards Th-2 response. If signaling between trophoblastic cells and NK cells remains poor it causes insufficient trophoblast invasion and defective spiral artery remodeling in early pregnancy. This may lead to hypoxic and dysfunctional placenta, placental infarction and thrombosis, and finally, generalized inflammation, in which systemic endothelial dysfunction is an essential component (Matthiesen et al. 2005, Redman and Sargent. 2005). This suggests that placental abruption may result from placentation failure caused by flawed maternal immune response to paternal antigens (Baumann et al. 2000). An excessive activation of the immune system in placental abruption may suggest past exposure to strong superantigens (Steinborn et al. 2004).

Inflammation

Placental abruption may be a manifestation of acute or chronic inflammatory process (Ananth et al. 2006a). Infections and tissue injury cause a rapid release of various bioactive mediators at the maternal-fetal interface (Nakatsuka et al. 1999, Ananth et al. 2006a). Neutrophils and macrophages are increased in placentas of women with abruption compared to controls (Ananth et al. 2006b). Oxidative stress and products of vascular activation and coagulation such as thrombin may have similar effects (Ananth et al. 2006a). Abruption is associated with a thrombin-enhanced expression of interleukin (IL)-8, a potent neutrophil chemoattractant, which leads to a marked infiltration of decidual neutrophils (Rosen et al. 2002). Increased production of proinflammatory cytokines such as tumor necrosis factor (TNF)- α and IL- β 1 can stimulate the production of matrix metalloproteinases (MMP) by trophoblasts and other cell types (Ananth et al. 2006a). Increased premature production of MMP may result in the destruction of the extracellular matrix and cell to cell interactions that lead to premature detachment (Ananth et al. 2006a). MMPs seem to play important roles in normal placental detachment (Ananth et al. 2006a). Reduced MMP activity is known to be associated with retained placentas in animals (Maj and Kankofer. 1997). In a recent study 51% of women with preterm abruption (<37 weeks)

and 44% of women with term abruption (≥ 37 weeks) had acute inflammation-associated condition or chronic clinical process, compared to 37% of control women with preterm delivery and 25% of control women with term delivery (Ananth et al. 2006a).

C-reactive protein (CRP) is an objective and sensitive marker of infection and inflammation (Kluft and de Maat. 2002, Pitiphat et al. 2005). The levels and kinetics of CRP in cases of placental abruption have not been studied, although CRP has been implicated in many other pregnancy complications such as pre-eclampsia, gestational diabetes and preterm delivery with or without chorioamnionitis (Loukovaara et al. 2003, Qiu et al. 2004a, Qiu et al. 2004b, Pitiphat et al. 2005). Chlamydiae are common pathogens linked to chronic inflammatory disease (Paavonen and Eggert-Kruse. 1999, Hammerschlag. 2007, Meyers et al. 2007). *C. pneumonia* antibodies have been increased in women with preeclampsia in some (Heine et al. 2003, Goulis et al. 2005) but not all studies (Teran et al. 2003, Raynor et al. 2004). *C. trachomatis* has been linked to several adverse pregnancy outcomes (McGregor and French. 1991, Claman et al. 1995, Gencay et al. 2000, Karinen et al. 2005). However, there is no data concerning placental abruption and chlamydiae.

Vascular disease

Normal placentation requires trophoblast invasion of maternal spiral arteries, and development of a high-flow, low-resistance uteroplacental circulation (Eskes. 1997). Vascular remodeling occurs under the influence of several proangiogenic and antiangiogenic factors (Zygmunt et al. 2003, Lambert-Messerlian and Canick. 2004, Lam et al. 2005, Levine and Karumanchi. 2005, Redman and Sargent. 2005). The former factors, i.e. placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), promote the formation of placental blood vessels and also the invasion of trophoblasts in the spiral arteries (Zygmunt et al. 2003, Lam et al. 2005, Redman and Sargent. 2005). Antiangiogenic factors include soluble fms-like tyrosine kinase 1 (sFlt-1) which binds biologically active forms of PlGF and VEGF (Levine and Karumanchi. 2005), and soluble endoglin (sEng) which blocks the binding of transforming growth factor isoforms to endothelial receptors (Venkatesha et al. 2006). In placental abruption the trophoblast invasion in the spiral arteries and consequent early vascularization is defective (Dommissse and Tiltman. 1992, Kraus et al. 2004). It appears that PlGF deficiency and sFlt-1 excess may result from placental hypoxia associated with incomplete remodeling of maternal spiral arteries. The incomplete remodeling of arteries causes high resistance to uterine artery blood flow which may predispose to vascular rupture in the placental bed leading to placental abruption (Dommissse and Tiltman. 1992, Eskes. 1997, Signore et al. 2006). This mechanism causes “a classic abruption” with arterial bleeding

and usually with more severe symptoms (Elliott et al. 1998, Hladky et al. 2002). Placental abruption can also be caused by a venous bleeding from marginal lakes around the edge of the placenta leading often to preterm birth (Elliott et al. 1998, Hladky et al. 2002).

Risk factors

Although many risk factors for placental abruption are well known, the cause for this serious complication often remains unexplained. Also, abruption often happens suddenly and is unexpected. The pathogenesis of placental abruption may be multifactorial and may vary in women with different risk factors. The known or suspected risk factors for placental abruption are summarized in Tables 1, 2 and 3 (Kåregård and Gennser.1986, Saftlas et al. 1991, Miller et al. 1995, Hemminki and Meriläinen. 1996, Ananth et al. 1997, Hulse et al. 1997, Kramer et al. 1997, Ananth et al. 1999b, Kupferminc et al. 1999, Ray and Laskin. 1999, Baumann et al. 2000, Kyrklund-Blomberg et al. 2001, Lydon-Rochelle et al. 2001, Pandian Z et al. 2001, Campbell and Templeton. 2004, Steegers-Theunissen et al. 2004, Ananth et al. 2005a, Casey et al. 2005, Shevel et al. 2005, Lindqvist and Happach. 2006, Robertson et al. 2006, Ananth and Cnattingius. 2007, Ananth et al. 2007b, Burd et al. 2007).

Smoking

Approximately 10-20% of women in industrialized countries smoke during pregnancy (Ananth and Cnattingius. 2007); in Finland the rate is approximately 15% (Stakes 2006). Smoking is a well known risk factor for placental abruption and also for many other adverse pregnancy outcomes, including infertility, spontaneous abortion, low birth weight, preterm delivery, and long term physical and developmental disorders in infants (Ananth et al. 1999a). The association with placental abruption and smoking was first reported in 1976 (Meyer et al. 1976). Approximately 5% of all perinatal deaths are attributable to maternal smoking largely due to placental abruption (Andres and Day. 2000). Smoking is also associated with a 2.5-fold increase in severe abruption resulting in fetal death (Raymond and Mills. 1993). Studies have shown that the relative risk for placental abruption associated with maternal smoking during pregnancy varies from 1.5 to 2.5 (Voigt et al. 1990 Ananth et al. 1999a, Tuthill et al. 1999, Mortensen et al. 2001, Ananth and Cnattingius. 2007) with a strong dose dependency (Kyrklund-Blomberg et al. 2001, Ananth and Cnattingius. 2007). However, there seems to be a threshold effect at approximately 10 cigarettes per day after which the risk remains relatively constant (Ananth et

al. 1999a). Also, the duration of smoking is associated with an increasing incidence of placental abruption (Naeye. 1980) although the risk is largely confined to the current pregnancy (Ananth and Cnattingius. 2007). Quitting smoking before pregnancy or early in pregnancy reduces the risk of abruption to the level of nonsmokers (Naeye. 1980, Andres and Day. 2000, Ananth and Cnattingius. 2007). This suggests that the adverse effects of maternal smoking are largely due to a direct toxic effect of smoking during pregnancy (Ananth and Cnattingius. 2007).

Although the mechanisms explaining the association between smoking and placental abruption remain largely speculative, it is known that smoking increases homocysteine levels in the plasma, and this may play a role (Ray and Laskin. 1999). Hyperhomocysteinemia can induce endothelial cell injury and dysfunction leading to local thromboembolism and defects within the placental vascular bed (de Vries et al. 1997). Also, the direct effect of smoking on placental abruption may be mediated through vasoconstrictive effects of nicotine on uterine and umbilical arteries as well as carboxyhemoglobin which interferes with oxygenation. Nicotine and carbon monoxide (CO) cross the placenta. The levels of nicotine and CO in the fetal circulation are 15% higher than those in blood (Luck et al. 1985, Andres and Day. 2000). The concentrations of nicotine amniotic fluid can be 88% higher than in maternal plasma (Luck et al. 1985). Nicotine decreases the flow in uterine and umbilical arteries causing changes in the fetal oxygenation and acid-base balance. Fetal heart rate decreases and mean arterial pressure increases (Andres and Day. 2000). CO binds to hemoglobin to form carboxyhemoglobin. Also this agent decreases fetal oxygenation (Andres and Day. 2000). The hypoxic changes caused by nicotine and CO can lead to placental infarcts, common among smokers, suggesting that increased capillary fragility might result in arterial rupture leading to placental abruption (Naeye. 1980, Kaminsky et al. 2007). In placentas of smoking women the perivillous knotting in syncytiotrophoblasts may be caused by an attempt by the villi to increase surface area through angiogenesis and neovascularization (Kaminsky et al. 2007). Placental function is impaired although placental weight is increased in smoking women which may be due to adaptive angiogenesis in peripheral villous tree (Pfarrer et al. 1999). This is reflected by increased levels of proangiogenic PlGF and reduced levels of antiangiogenic sEng and sFlt-1 (Levine et al. 2006). Smokers also have lower concentrations of cellular fibronectin (Lain et al. 2003), which connects trophoblast to the uterine decidua at the site of implantation (Eskes. 1997).

According to a meta-analysis 15% to 25% of placental abruption episodes may be attributable to cigarette smoking (Ananth et al. 1999a). Thus, a considerable proportion of placental abruption episodes could be prevented if women quit smoking during pregnancy. No data exist of spouse smoking and placental abruption.

Hypertensive complications

Hypertensive disorders in pregnancy, i.e. chronic hypertension, chronic hypertension with superimposed preeclampsia, pregnancy induced hypertension (PIH), and preeclampsia have all been found to be risk factors for placental abruption in many but not all studies (Ananth et al. 1996, Ananth et al. 1997, Kramer et al. 1997, Ananth et al. 1999a, Ananth et al. 2007b). Comparison of these studies is problematic since definitions vary remarkably.

Chronic hypertension complicates 0.3-0.8% of pregnancies and increasing maternal age and parity increase the risk (Ananth et al. 2007b). Smoking and the black race increase the risk (Ananth et al. 2007b). In some (Ananth et al. 1996, Kramer et al. 1997, Ananth et al. 2007b) but not all (Ananth et al. 1997) studies chronic hypertension has been a risk factor for placental abruption. In one study the rate of abruption among women with or without chronic hypertension was 1.56 % and 0.6 % in singleton pregnancies, respectively (Ananth et al. 2007b). After adjustment for potential confounders women with chronic hypertension were at 2.4-fold increased risk for abruption (Ananth et al. 2007b). In another study women with chronic hypertension had no increased risk for abruption (RR 1.4; 95% CI 0.5-3.6) (Ananth et al. 1997). Although chronic hypertension alone has not been a risk factor for placental abruption in all studies, chronic hypertension with superimposed preeclampsia has increased the risk for placental abruption 2.8- to 7.7-fold in several studies (Ananth et al. 1997, Ananth et al. 2007b).

Severe preeclampsia is a strong risk factor for placental abruption (Ananth et al. 1997, Ananth et al. 1999a). However, PIH and mild preeclampsia are risk factors for placental abruption in some (Kramer et al. 1997) but not all studies (Ananth et al. 1997, Ananth et al. 1999a). Comparison of the studies is difficult due to different criteria used for preeclampsia (Ananth et al. 1996, Ananth et al. 1997, Kramer et al. 1997, Ananth et al. 1999a). The risk for abruption is further increased among women who have hypertensive disorder and who smoke (Ananth et al. 1999a). In two previous Finnish studies chronic hypertension or PIH showed borderline association with placental abruption (Ylä-Outinen et al. 1987, Toivonen et al. 2002). One of the two studies found strong association between preeclampsia and placental abruption (Toivonen et al. 2002).

Hyperhomocysteinemia and thrombophilia

Homocysteine is an intermediate product in the metabolism of the essential amino acid methionine (Steegers-Theunissen et al. 2004). Homocysteine is methylated to methionine and this metabolism involves 5,10-methylenetetrahydrofolate reductase (MTHFR), folate, vitamins B6 and B12 (Ray and Laskin. 1999, Eskes. 2001). Hyperhomocysteinemia induces endothelial cell injury and dysfunction and leads to atherosclerosis and thromboembolism (de Vries et al.

1997). There is an association between hyperhomocysteinemia and placental abruption (Goddijn-Wessel et al. 1996, de Vries et al. 1997, Ray and Laskin. 1999, Vollset et al. 2000, Steegers-Theunissen et al. 2004). The association is stronger with shorter interval between sampling and delivery (Vollset et al. 2000) but the time of testing should be at least > 10 weeks postpartum (de Vries et al. 1997). Hyperhomocysteinemia is a strong indicator of folate and B12 deficiency (Ray and Laskin. 1999). According to a meta-analysis folate deficiency may also be a risk factor for placental abruption (OR 25.9, 95% CI 0.9-736.3) (Ray and Laskin. 1999). In another study, high red cell folate decreased the risk for placental abruption (Steegers-Theunissen et al. 2004). In some studies, but not all, vitamin B12 deficiency has been a risk factor for placental abruption (Ray and Laskin. 1999, Steegers-Theunissen et al. 2004). Young women with folate deficiency and hyperhomocysteinemia may be prone to endothelial dysfunction including placental vasculature (Ray and Laskin. 1999). Although plasma homocysteine levels can be lowered by administration of vitamin B6 and folate (Eskes. 2001), older large prospective studies have failed to show any association between folate supplementation and placental abruption (Konje and Taylor. 2001). However, a recent Norwegian study showed that women who used folic acid or multivitamin supplements during pregnancy had 26% lower risk of developing placental abruption than women who had not used such supplements (Nilsen et al. 2008).

It is known that inherited and acquired thrombophilias increase the risk of venous thromboembolism and adverse pregnancy outcome, i.e. early pregnancy loss, preeclampsia, intrauterine growth restriction (IUGR), stillbirth, or placental abruption (Robertson et al. 2006, Ulander et al. 2006). One of the early studies found that 65% of women with preeclampsia, IUGR, unexplained stillbirth, or placental abruption had heritable or acquired thrombophilia (Kupferminc et al. 1999). The risk found in individual studies has varied due to different study designs (Robertson et al. 2006). Thrombophilias associated with abruption include MTHFR deficiency, factor V Leiden mutation, prothrombin gene mutation, protein S and protein C deficiency, antithrombin deficiency, lupus anticoagulant, and anticardiolipin antibodies (Oyelese and Ananth. 2006). Homozygous MTHFR point mutation 677 C to T transition has been associated with placental abruption in several (Ray and Laskin. 1999, Eskes. 2001, Nurk et al. 2004) but not all studies (Kupferminc et al. 1999, Jääskeläinen et al. 2006). Some studies have shown an association between placental abruption and heterozygous factor V Leiden mutation (Kupferminc et al. 1999, Facchinetti et al. 2003, Robertson et al. 2004). However, in a Finnish study M385T polymorphism in the factor V gene, but not Leiden mutation, was associated with placental abruption (Jääskeläinen et al. 2004). A Swedish study of 102 women with abruption

also failed to show any difference in factor V Leiden carrier rate between cases and controls (Prochazka et al. 2003). The rate of heterozygous prothrombin gene mutation is increased 8- to 9-fold among women with placental abruption (Kupferminc et al. 1999, Kupferminc et al. 2000). There is insufficient data of other thrombophilias and placental abruption (Robertson et al. 2006). The combination of hyperhomocysteinemia and thrombophilia increases the risk of placental abruption 3- to 7-fold (Eskes. 2001).

Chorioamnionitis

Clinical diagnosis of chorioamnionitis may be difficult (Smulian et al. 1999) and can only be confirmed histologically (Smulian et al. 1999). However, micro-organisms are isolated in only 70% of placentas with histologic chorioamnionitis (Smulian et al. 1999). In some cases histologic inflammation may be due to a variety of noninfectious causes such as fetal hypoxia, amniotic fluid pH changes, immunologic responses to fetal tissues, and meconium (Smulian et al. 1999). Chorioamnionitis may precede abruption or abruption may precede chorioamnionitis, or the two conditions may be unrelated and present simultaneously (Darby et al. 1989). Direct bacterial colonization of the decidua with tissue inflammation may initiate a process that results ultimately in placental abruption (Darby et al. 1989). Sometimes a subclinical decidual thrombosis may initiate an inflammatory process (Darby et al. 1989). Nevertheless, infection activates cytokines such as IL and TNF. These cytokines upregulate the production and activity of MMPs in the trophoblast (Nath et al. 2007). This may result in destruction of the extracellular matrix and cell to cell interactions which then may lead to disruption of the placental attachment and finally to placental abruption (Nath et al. 2007).

Chorioamnionitis occurs three to seven times more likely in patients with abruption than in controls (Darby et al. 1989, Saftlas et al. 1991). In a recent study the rate of histologically confirmed chorioamnionitis among women with placental abruption was 30% (Nath et al. 2007). Severe chorioamnionitis was strongly associated with placental abruption both in term and preterm pregnancies (Nath et al. 2007). In another study, the rates of abruption among women with or without intrauterine infection were 4.8% and 0.8% (Ananth et al. 2004). The attributable proportion of intrauterine infections among all abruptions was 6.7% (Ananth et al. 2004).

Premature rupture of membranes

Preterm premature rupture of membranes (PROM) occurs in 3% of pregnancies and is responsible for one third of all preterm births (Mercer. 2003). Approximately 4-12 % of patients with preterm PROM develop placental abruption (Ananth et al. 1996, Mercer. 2003). The risk of

this complication increases with decreasing gestational age at membrane rupture (Mercer. 2003). Women exposed to prolonged preterm PROM are at increased risk of developing abruption if the latency between the time of membrane rupture and delivery exceeds 24 hours (Ananth et al. 2004).

Preterm PROM is often associated with ascending intrauterine infection. Recent evidence has linked neutrophil infiltration in the decidua with preterm PROM and placental abruption. The risk of abruption is 3.6-fold higher among women with preterm PROM, compared to women with intact membranes (Ananth et al. 2004). When preterm PROM is accompanied with intrauterine infection, the risk of abruption is 9-fold higher, compared to women with intact membranes and no infection (Ananth et al. 2004). Although preterm PROM frequently precedes abruption, sometimes placental abruption may lead to PROM (Rosen et al. 2002). Abruption leads to marked infiltration of neutrophils in the decidua (Rosen et al. 2002). This influx of neutrophils is a rich source of proteases that can degrade extracellular matrix, leading to preterm PROM. Therefore, it is difficult to determine whether neutrophil infiltration into the decidua is secondary to vascular disruption or whether it is the primary cause of abruption (Nath et al. 2007). In some women with preterm PROM reduction of uterine volume may lead to placental abruption (Ananth et al. 1996).

Trauma

Physical trauma complicates 6-7 % of pregnancies (Pak et al. 1998, Schiff and Holt. 2002). Of these, motor vehicle accidents account 66%, falls and assaults 33% (Pak et al. 1998). Domestic violence is included in assaults and has been reported in 8-20 % of cases (Helton et al. 1987, Parker et al. 1994, Rachana et al. 2002). Adverse pregnancy outcome after minor trauma occurs in 1-5% of cases (Pak et al. 1998). Placental abruption is attributable to any trauma in approximately 6% of all cases (Pearlman et al. 1990) and to major trauma in 20-25% of cases (Vaizey et al. 1994), but is difficult to predict on the basis of the severity of the injury (Pearlman et al. 1990). This makes placental abruption the second most common cause of fetal loss after maternal death in pregnant trauma patients (Henderson and Mallon. 1998). The mechanism of abruption in trauma cases is directly related to the injury. The relatively elastic uterus is able to alter its shape in reaction to forces applied to the abdomen, whereas the less elastic placenta is not. A shearing effect is therefore created, disrupting the attachment of placenta to the decidua (Kingston et al. 2003). Placental abruption usually becomes manifest within 6 to 48 hours after injury but can occur up to 5 days later (Higgins and Garite. 1984, Pearlman et al. 1990, Curet et

al. 2000). Placentas that are anteriorly placed are at increased risk for fetomaternal transfusion (Pearlman et al. 1990).

External cephalic version is also associated with placental abruption although the risk is low. In a recent review the incidence of placental abruption due to external cephalic version was only 0.12% (Collaris and Oei. 2004).

Others

Other *prepregnancy risk factors* for placental abruption include previous cesarean section (C/S) and uterine anomaly (Green. 1989, Hemminki and Meriläinen. 1996). Also, the risk for placental abruption is increased in the next pregnancy followed by birth of a small for gestational age (SGA) newborn, premature birth, PIH, preeclampsia, or stillbirth (Rasmussen et al. 1999, Lindqvist and Happach. 2006, Ananth et al. 2007a). This may indicate a common etiologic factor for these conditions (Rasmussen et al. 1999). Both short and long interpregnancy intervals have also been associated with increased risk of placental abruption (Rasmussen et al. 1999). According to some studies cesarean first delivery increases the risk for placental abruption by 30-40% in the next pregnancy when compared to women with vaginal first delivery (Rasmussen et al. 1999, Lydon-Rochelle et al. 2001, Getahun et al. 2006, Yang et al. 2007). According to a Finnish study the risk is even higher, i.e. 2.4-fold among primiparous and 3.9-fold among multiparous women (Hemminki and Meriläinen. 1996). If the interpregnancy interval is less than one year the risk of abruption is increased by 52% in women with vaginal first delivery and by 111% in women with cesarean first delivery (Getahun et al. 2006). Uterine low segment scar may impair placental attachment, and therefore increase the risk for abruption (Rasmussen et al. 1999, Lydon-Rochelle et al. 2001).

Although mentioned in some textbooks (Green. 1989), the most recent studies have not demonstrated any association between placental abruption and congenital uterine malformation. Abnormal fusion of the Müllerian ducts causes varying degrees of uterine anomalies (Heinonen et al. 2000) present in 0.1-2% of all women (Acien. 1997). It may be that uterine malformation leads to poor decidualization and placentation at the site of implantation. Also the contractibility of malformed uterus may be disturbed or uncoordinated increasing the risk for placental abruption (Dabirashrafi et al. 1995).

Other *pregnancy related risk factors* for placental abruption are placenta previa, bleeding during pregnancy, multiple pregnancy, and alcohol and cocaine use (Kaminski et al. 1976, Sipilä et al. 1992, Miller et al. 1995, Baumann et al. 2000, Ananth et al. 2001, Salihu et al. 2005).

Placenta previa is a notable risk factor for placental abruption (Konje and Taylor. 2001) although not all studies confirm this (Oyelese and Ananth. 2006). Approximately 10% of women with placenta previa have coexisting abruption (Konje and Taylor. 2001). In one study of the risk factors for placental abruption, uterine bleeding > 28 gestational weeks and placenta previa were the strongest predictors (Baumann et al. 2000). Among women with placenta previa the risk was 3- to 4-fold, and among women with uterine bleeding > 28 weeks the risk was 12- to 19-fold (Baumann et al. 2000). If women had uterine bleeding at < 28 weeks the risk for placental abruption was 2-fold (Baumann et al. 2000). Bleeding in early pregnancy carries an increased risk for abruption in later pregnancy (Ananth et al. 2006b). The presence of a subchorionic or retroplacental hematoma in the first trimester ultrasound examination increases the risk for subsequent placental abruption 6- to 11-fold (Ball et al. 1996, Nagy et al. 2003). This may reflect a hematoma impairing normal placentation. On the other hand, a hematoma can result from impaired placentation (Nagy et al. 2003).

The risk of placental abruption is 2- to 3-fold in twin pregnancies compared to singleton pregnancies (Baumann et al. 2000, Ananth et al. 2001, Campbell and Templeton. 2004, Salihu et al. 2005) although not all studies have confirmed this (Kramer et al. 1997). With increasing multiplicity the likelihood of placental abruption increases but associated perinatal mortality decreases (Salihu et al. 2005). The risk of preterm birth or SGA in twin pregnancies with placental abruption is higher than among twin pregnancies without placental abruption (Ananth et al. 2005b). The discordant growth of twins is a risk factor for placental abruption (Ananth et al. 2003). The risk factor profiles for placental abruption seem to be different among singleton births and twin births (Ananth et al. 2001). The abruption in multifetal pregnancies may have a different mechanism (Ananth et al. 2001, Salihu et al. 2005).

Alcohol use during pregnancy is a known risk factor for fetal neurodevelopmental abnormalities, several fetal malformations, and SGA (Kaminski et al. 1976, Halmesmäki. 1988, Sokol et al. 2003). Alcohol easily crosses placenta and may disturb the hormonal balance in the mother and fetus (Gabriel et al. 1998). No safe amount of alcohol consumption during pregnancy has been determined. In one study, the risk of stillbirth was higher among alcohol users, particularly due to placental abruption (Kaminski et al. 1976). In another study, the risk for placental abruption did not vary according to alcohol consumption (Kramer et al. 1997).

In the United States, the incidence of cocaine ingestion during pregnancy has been reported as high as 10% in selected populations (Miller et al. 1995, Baumann et al. 2000). The risk for placental abruption among cocaine users is 3.9- to 8.6-fold (Miller et al. 1995, Hulse et al. 1997) and may result from vasoconstrictive effects of cocaine (Hladky et al. 2002). Although the

relationship between placental abruption and cocaine use is confounded by other risk factors, including use of other drugs, tobacco, and lack of prenatal care, cocaine use remains as an independent risk factor (Hladky et al. 2002). Amphetamine use is also associated with placental abruption probably due to similar mechanisms as cocaine use (Kuczkowski. 2003).

Table 1. Sociodemographic and behavioural risk factors for placental abruption based on published data. Odds ratio (OR) given if available

Risk factor	OR
Sociodemographic	
Maternal age ≥ 35 years	1.3-2.6
Maternal age <20 years	0.8-1.5
Parity ≥ 3	1.0-1.6
Black race	1.9
White race	1.2
Lower socio-economic status	
Unmarried or single mother	1.5-6.8
Behavioral	
Cigarette smoking	1.5-2.5
Alcohol use	2.8-3.4
Cocaine use	3.9-8.6
Trauma	17.3
Unexplained infertility or infertility treatments	1.3-2.4

Table 2. Maternal and historical risk factors for placental abruption based on published data. Odds ratio (OR) given if available

Risk factor	OR
Maternal	
Chronic hypertension	1.4-2.4
Hyperhomocysteinemia	1.8-5.3
Thrombophilia	1.4-7.7
Folate deficiencies	25.9
Diabetes mellitus (all types)	0.8-2.8
Hypothyreosis	3.0
Anemia	2.2-2.8
Uterine anomaly	
Uterine tumor	0.8-2.8
History of	
Cesarean section	1.3-3.9
Miscarriage	1.4-3.2
Preeclampsia	1.9
Stillbirth	13.1
Placental abruption	10.2-25.8

Table 3. Pregnancy associated risk factors for placental abruption based on published data. Odds ratio (OR) given if available

Risk factor	OR
Pregnancy induced hypertension	0.9-1.6
Preeclampsia	1.9-3.8
Superimposed preeclampsia	2.8
Chorioamnionitis	1.2-2.6
Premature rupture of membranes	0.9-5.9
Oligohydramnion	1.0-2.8
Polyhydramnion	3.0-3.2
Placenta previa	3.2-4.3
Vaginal bleeding \leq 28 gestational weeks	2.0-2.2
Vaginal bleeding \geq 28 gestational weeks	12.3-18.7
Multiple gestation	2.0-2.9
External version	
Male fetal gender	1.3
Small for gestational age fetus	1.3-4.1
Short umbilical cord	1.3-2.0
Velamentous umbilical cord insertion	1.8-3.7
Vena cava syndrome	

Clinical presentation and diagnosis

Although the symptoms of placental abruption are typical and have been well described, they can vary considerably from one patient to another (Baron and Hill. 1998).

Symptoms

Classic symptoms of placental abruption are vaginal bleeding, abdominal pain, uterine contractions and tenderness (Baron and Hill. 1998). All of these symptoms are not invariably present, and asymptomatic presentation does not exclude placental abruption (Baron and Hill. 1998, Oyelese and Ananth. 2006). The symptoms and their severity depend on the location of abruption, whether it is revealed or concealed, and the degree of abruption (Oyelese and Ananth. 2006). Vaginal bleeding is present in 70-80% of cases (Baron and Hill. 1998, Konje and Taylor. 2001) and its amount correlates poorly with the degree of abruption (Oyelese and Ananth. 2006). If the membranes are ruptured, blood stained amniotic fluid leaks into vagina (Konje and Taylor. 2001). Uterine tenderness or pain is present in 66% and tonic uterine contractions in 34% (Baron and Hill. 1998). The presence of pain probably indicates extravasation of blood into the myometrium (Konje and Taylor. 2001). The contractions are unusually frequent with a rate of

more than five in 10 minutes (Konje and Taylor. 2001, Oyelese and Ananth. 2006). The presence of uterine contractions may, however, be difficult to distinguish from general abdominal pain associated with abruption. Abdominal pain is less common in posterior placentas (Konje and Taylor. 2001).

Clinical signs

Four grades (0-3) of placental abruption have been described (Table 4) (adapted from Konje and Taylor. 2001), the most severe form occurring in about 0.2% of pregnancies (Konje and Taylor. 2001).

Table 4. Grading of placental abruption

Grade	Description
0	Asymptomatic, a small retroplacental clot
1	Vaginal bleeding, uterine hypertonus and tenderness may be present; no signs of maternal or fetal distress
2	Vaginal bleeding possible, no signs of maternal shock; signs of fetal distress present
3	Vaginal bleeding possible, uterine hypertonus, “wooden-hard” uterus on palpation, persistent abdominal pain, maternal shock and fetal death, coagulopathy in 30% of cases

Placental abruption is often confirmed by gross examination of delivered placenta. In recent abruption the inspection of placenta demonstrates a crater-like depression on the maternal surface of the placenta covered by dark clotted blood, so called “delle” (Eskes. 1997). In older abruptions fibrin deposits appear on the site of abruption (Oyelese and Ananth. 2006). A totally abrupted placenta may not differ on the maternal surface from a normal placenta at delivery (Eskes. 1997).

Bleeding may occur into the uterine myometrium, leading to a purple colored uterus, so called Couvelaire uterus (Oyelese and Ananth. 2006) Such an uterus contracts poorly which can result in postpartum hemorrhage (Konje and Taylor. 2001).

Ultrasound

If placental abruption is suspected based on clinical symptoms, ultrasound examination is often performed in an attempt to visualize the extent of subchorionic or retroplacental hematoma. In some cases, placental abruption may be detected based on ultrasonographic findings even in

asymptomatic patients (Oyelese and Ananth. 2006). The ultrasonographic appearance of abruption depends on the size and location as well as the age of the hematoma (Nyberg et al. 1987). The appearance of hematoma in the acute phase of abruption is from hyperechoic to isoechoic when compared with the placenta. When the hematoma resolves it becomes more hypoechoic within 1 week and sonolucent within 2 weeks (Nyberg et al. 1987). Small abruptions or acute revealed abruptions are often not detectable by ultrasound (Oyelese and Ananth. 2006). Concealed hemorrhage may be more easily seen by ultrasound (Glantz and Purnell. 2002). Despite improvement in sonographic equipments the sensitivity of the diagnosis of abruption has not improved (Glantz and Purnell. 2002). In one study ultrasound correctly diagnosed abruption only in 25% of cases (Glantz and Purnell. 2002). When a clot was visualized by ultrasound, the positive predictive value for abruption was 88% (Glantz and Purnell. 2002). Also, when a subchorionic or retroplacental hematoma was identified by ultrasound the management was more aggressive and perinatal outcome was worse (Glantz and Purnell. 2002). It may be that positive sonographic findings with more severe abruption lead to unnecessary intervention which impairs neonatal outcome mainly due to prematurity (Glantz and Purnell. 2002). Although ultrasound is not accurate in the diagnosis of abruption it is useful in monitoring cases managed expectantly and in excluding coincident placenta previa (Konje and Taylor. 2001).

Cardiotocographic changes

In severe cases of placental abruption the fetus presents with heart rate abnormalities. A variety of fetal cardiotocographic (CTG) patterns have been described in association with placental abruption and fetal distress, and may include repetitive late or variable decelerations, decreased beat-to-beat variability, bradycardia, or sinusoidal fetal heart rate pattern (Oyelese and Ananth. 2006). Abnormal CTG in association with placental abruption predicts poor fetal outcome, even death (Manolitsas et al. 1994). On the other hand, conservative expectant management seems to be safe in preterm pregnancies with placental abruption and normal CTG (Manolitsas et al. 1994).

Placental histopathology

Histopathology of abrupted placentas often shows evidence of acute and chronic lesions (Ananth et al. 2006b). Acute lesions include neutrophil infiltration of the chorionic plate and chronic lesions include placental infarcts in the decidua (Ananth et al. 2006b). Chronic lesions develop due to a lack of adequate trophoblastic invasion (Dommissie and Tiltman. 1992). Histological signs of chorioamnionitis and deciduitis with neutrophil infiltration are associated with placental

abruption in one third of the cases (Kaminsky et al. 2007, Nath et al. 2007). Acute atherosclerosis in spiral arteries leads to distinctive necrotizing decidual lesions (Eskes. 1997, Ananth et al. 2006b) ultimately leading to vascular thrombosis, placental infarcts and fibrin deposits (Darby et al. 1989, Eskes. 1997, Kaminsky et al. 2007).

Intervillous thrombosis results from intraplacental hemorrhage from villous capillaries and is associated with chorionic villous hemorrhage. Intervillous thrombosis is more common in smoking women with placental abruption (Kaminsky et al. 2007). This may further reduce uteroplacental and fetal blood flow leading to chronic underperfusion. Chronic hypoxia is manifested by increased villous fibrosis and trophoblast knotting (Kaminsky et al. 2007). One study found that necrosis in the decidua basalis at the margin of the placenta was most frequent in smoking women suggesting that such necrosis could initiate placental abruption (Naeye. 1980).

Management

The management of placental abruption depends on the extent of abruption, gestational age, and maternal and fetal condition. The management should be individualized (Oyelese and Ananth. 2006). Severe abruption with intrauterine fetal death, regardless of gestational age, should be managed by vaginal delivery if there are no contraindications, (Konje and Taylor. 2001, Oyelese and Ananth. 2006). Labor usually progresses rapidly due to continuous contractions (Hladky et al. 2002) and if not, amniotomy can be performed. Augmentation of uterine contractions by oxytocin infusion or ripening of cervix by prostaglandins must be done cautiously as the risk of uterine rupture may exist in placental abruption (Konje and Taylor. 2001). Concealed bleeding into the myometrium, maternal tachycardia, or hypertension may lead to underestimation of the blood loss (Konje and Taylor. 2001). Intravenous cannula should be inserted and blood products and coagulation factors given if necessary (Hladky et al. 2002, Oyelese and Ananth. 2006). When labor does not progress rapidly or mother is unstable C/S may be necessary to avoid worsening of the coagulopathy (Hladky et al. 2002, Oyelese and Ananth. 2006). DIC is present in approximately 35% of cases of severe placental abruption (Konje and Taylor. 2001). The patient should be monitored closely after vaginal or operative delivery since severe hemorrhage occurs in 25% of the cases (Konje and Taylor. 2001). Hysterectomy may occasionally be necessary (Konje and Taylor. 2001, Oyelese and Ananth. 2006).

If the fetus is alive and pregnancy near term, prompt delivery is indicated. In cases of fetal or maternal compromise, cesarean delivery should be performed. If both fetal and maternal conditions are reassuring, vaginal delivery is reasonable. Established labor should be allowed to progress, otherwise induction of labor should be considered (Oyelese and Ananth. 2006). If abruption is suspected on the basis of an incidental finding on ultrasound in a term pregnancy, vaginal delivery is indicated (Hladky et al. 2002, Oyelese and Ananth. 2006).

Partial placental abruption at 20-34 weeks of gestation may be managed conservatively if maternal and fetal conditions are reassuring. Patient must be closely monitored and fetal growth followed (Konje and Taylor. 2001, Hladky et al. 2002, Oyelese and Ananth. 2006). At 24 to 34 weeks, steroids to promote fetal lung maturation should be given. It may be possible to discharge these patients if fetal condition is reassuring after patients have remained stable for several days (Hladky et al. 2002, Oyelese and Ananth. 2006). If the bleeding episodes are recurrent but fetal condition is satisfactory, induction is recommend at 37-38 gestational weeks (Konje and Taylor. 2001). Tocolytics such as β -sympathomimetics, atosiban, or magnesium sulfate can be used in selected cases of preterm placental abruption (Hladky et al. 2002).

Pregnant women should be followed for a minimum of 4 hours after abdominal or other trauma. If uterine contractions, vaginal bleeding, or fetal heart rate changes occur the follow-up should be extended (Oyelese and Ananth. 2006). All rhesus negative patients with placental abruption should receive anti-D immunoglobulin within 72 hours (Konje and Taylor. 2001).

Prediction

It is likely that in the majority of cases placental abruption is a long-standing process dating back to the first trimester (Oyelese and Ananth. 2006). Therefore, it would be of clinical importance if this condition could be predicted before it manifests clinically. Many clinical variabes and findings have been studied to predict the risk of placental abruption.

Family history

There is some evidence that placental abruption could occur in families (Toivonen et al. 2004a, Lindqvist and Happach. 2006). According to one study placental abruption appears to cluster in families with an index patient with recurrent placental abruption (Toivonen et al. 2004a). According to another study 5% of women with abruption report first degree relatives with this complication (Lindqvist and Happach. 2006).

The high prevalence of thrombophilia among women with placental abruption supports the idea of genetic background (Robertson et al. 2006). In one study 20% of women with placental abruption reported first degree relatives with venous thrombosis compared to only 6.7% of the controls (Prochazka et al. 2003). Genetic studies involving the use of candidate gene approach have shown a positive association between placental abruption and polymorphisms of the nitric oxide synthase gene (Yoshimura et al. 2001, Hillermann et al. 2005) although this was not confirmed in the Finnish study (Toivonen et al. 2005). Also, it has been shown that low-activity haplotype of the microsomal epoxide hydrolase gene is protective against placental abruption (Toivonen et al. 2004b).

History of placental abruption

Perhaps the most accurate predictor of abruption is a placental abruption in prior pregnancy (Oyelese and Ananth. 2006). Women with a history of abruption have 7- to 20-fold risk of abruption in a subsequent pregnancy (Kåregård and Gennser. 1986, Ananth et al. 1996, Rasmussen et al. 1997) and this risk elevation is not related to smoking habits (Ananth and Cnattingius. 2007). Placental abruption recurs in 3-17% after one episode and in 19-25% after two episodes (Kåregård and Gennser. 1986, Ananth et al. 1996, Rasmussen et al. 1997, Rasmussen et al. 2000, Konje and Taylor. 2001, Toivonen et al. 2004).

Women with unexplained abruption may benefit from screening for thrombophilias and hyperhomocysteinemia (de Vries et al. 1997, Eskes. 2001, Oyelese and Ananth. 2006). Women who screen positive for thrombophilia can be treated with heparin and aspirin in subsequent pregnancies or with folate, vitamin B6, and B12 in the case of MTHFR deficiency or hyperhomocysteinemia, although no clear benefit has been demonstrated (Eskes. 2001, Oyelese and Ananth. 2006). Smoking should be stopped (Oyelese and Ananth. 2006). Because patients with abruption have an increased risk of impaired uteroplacental perfusion in subsequent pregnancies, serial growth scans every 4 weeks should be considered during the second half of the pregnancy (Rasmussen et al. 2000, Rasmussen et al. 2001, Oyelese and Ananth. 2006). In women with two or more prior abruptions, amniocentesis for lung maturity and delivery at about 37 weeks of gestation is recommended (Oyelese and Ananth. 2006).

Uterine artery flow measurement

Doppler evaluation of uterine arteries in the first and second trimester might be a useful screening tool in the prediction of preeclampsia and SGA (Harrington et al. 1996, Martin et al. 2001, Papageorgiou et al. 2001, Madazli et al. 2005, Pilalis et al. 2007). There are also data to

suggest that high uterine artery pulsatility index at 11-14 weeks or notching of the uterine artery waveform at 20-24 gestational weeks could predict subsequent abruption (Harrington et al. 1996, Pilalis et al. 2007) but these methods have not been universally accepted in the prediction of placental abruption.

Biochemical markers

Elevated maternal serum alpha-fetoprotein (AFP) is associated with structural fetal anomalies, including open neural tube defects, abdominal wall defects and congenital nephrosis (Chandra et al. 2003). Yet, approximately 1% of patients have an elevated AFP level that cannot be accounted for incorrect dates, structural or chromosomal abnormalities, or multiple gestation (Chandra et al. 2003). This unexplained second-trimester elevation in maternal serum AFP may be associated with subsequent adverse obstetric outcome including placental abruption (Katz et al. 1990, van Rijn et al. 1999, Chandra et al. 2003, Dugoff et al. 2005, Smith et al. 2006). Disruption at the fetal maternal interface permits transfer of AFP into the maternal circulation (Chandra et al. 2003). Chronic villitis and vascular lesions of thrombosis or infarction have been associated with elevated maternal serum AFP levels (Salafia et al. 1988). In a Finnish study, elevated AFP levels (> 2.0 MoM) were detected in 17% of pregnancies with subsequent placental abruption (Toivonen et al. 2002). In another study of women with preterm labor and placental abruption AFP levels were higher than in other groups with preterm labor (Bartha et al. 1997). Authors suggested that AFP could be used as a marker for placental abruption (Bartha et al. 1997), but so far this test has not been used for this purpose in clinical practise.

Maternal serum free beta human chorionic gonadotrophin (β -hCG) is commonly measured in the second trimester to screen for chromosomal abnormalities (van Rijn et al. 1999, Dugoff et al. 2005). In case of normal chromosomes this test may be elevated in pregnancy complications, such as in early fetal loss, preterm birth, PIH, preeclampsia, and SGA (Liu et al. 1999, van Rijn et al. 1999, Chandra et al. 2003, Dugoff et al. 2004, Dugoff et al. 2005). There are also some data linking high β -hCG levels to placental abruption (Liu et al. 1999). Abnormal increased levels of β -hCG may be due to decreased placental perfusion (Chandra et al. 2003). Theoretically, being purely a placental product (Liu et al. 1999, Chandra et al. 2003), β -hCG may hold promise for prediction of placental abruption.

Pregnancy-associated plasma protein A (PAPP-A), widely used in screening for chromosomal abnormalities at 10-14 weeks of gestation (Dugoff et al. 2004, Pilalis et al. 2007), is a protease for insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) (Dugoff et al. 2004). A recent cohort study of 34,271 women indicated that women with first-trimester PAPP-A levels at the

lowest fifth centile had an increased risk of placental abruption (Dugoff et al. 2004). In another study low PAPP-A was detected in 29% at the lowest fifth centile and 43% at the lowest tenth centile of women with placental abruption (Pilalis et al. 2007).

Proangiogenic placental growth factor (PlGF) and antiangiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) are the factors regulating placental angiogenesis throughout pregnancy. Presence and function of PlGF is critical during cytotrophoblastic invasion (Madazli et al. 2005). Soluble Flt-1 inhibits both PlGF and VEGF (Lam et al. 2005). Many studies have shown that the circulating levels of sFlt-1 are increased and those of PlGF and VEGF are decreased often weeks before onset of clinical preeclampsia (Levine et al. 2004, Thadhani et al. 2004, Signore et al. 2006, Wathen et al. 2006). It is also possible that smoking may affect these vasoregulators (Levine et al. 2006). In a recent study, decreased levels of PlGF and increased levels of sFlt-1/PlGF ratios at 21st to 32nd weeks of gestation preceded subsequent placental abruption but only in the women who also developed preeclampsia or PIH (Signore et al. 2006). Thus, it is not known whether placental abruption itself is associated with changes in these markers.

Maternal serum activin A is a glycoprotein (Keelan et al. 2002) which has many biological effects including tissue remodeling and regulation of trophoblast differentiation and invasion (Keelan et al. 2002, Madazli et al. 2005). Activin A is produced primarily by the placental trophoblast and its levels in maternal blood are elevated in preeclamptic patients (Madazli et al. 2005). One study reported two cases with placental abruption (without preeclampsia) who had high activin A levels weeks before placental abruption (Florio et al. 2003). This calls further studies on the value of this test in prediction of placental abruption.

Fibronectin is a glycoprotein synthesized in the endothelial cell (Madazli et al. 2005). With a glycopeptide domain it connects trophoblast to the uterine decidua at implantation site (Eskes. 1997). The levels of fibronectin are high in women with preeclampsia, perhaps as a result of endothelial damage (Madazli et al. 2005). The levels of fibronectin are also higher in women with placental abruption (Kanayama and Terao. 1992) but clearly more research is needed.

Thrombomodulin is a vascular endothelial cell receptor for thrombin which neutralizes thrombin clotting activity (Magriples et al. 1999). Thrombomodulin is a marker of endothelial cell damage and has been localized to the placental syncytiotrophoblast (Magriples et al. 1999). The levels of thrombomodulin may be elevated in women with placental abruption (Magriples et al. 1999).

Kleihauer-Betke test is not sensitive enough and should not be used to diagnose placental abruption (Emery et al. 1995, Magriples et al. 1999). *D-dimer*, a byproduct of clot lysis, has a high negative predictive value for thromboembolic events (Nolan et al. 1993, Adema and Gebert. 1995) and may also be used in early diagnosis of placental abruption (Nolan et al. 1993). Also

coagulation profile, i.e. *platelet count*, *prothrombin time*, *partial thromboplastin time*, and *fibrinogen level* have been used in the prediction of placental abruption (Nolan et al. 1993, Magriples et al. 1999) but their efficacy is not good enough for clinical purpose.

Risk factor analysis

Risk score analyses and mathematical modelling have been developed in order to predict placental abruption (Baumann et al. 2000, Lindqvist and Happach. 2006). An analysis of 52 obstetric risk factors related to placental abruption was used to create a mathematical model (Baumann et al. 2000). After multivariate analysis seven correlates overlapping between primiparous and multiparous women remained in the model. These were uterine bleeding at <28 and >28 gestational weeks, placenta previa, male fetal gender, preterm labor, breech position, and cigarette smoking. The strongest predictors of placental abruption were concomitant uterine bleeding at > 28 gestational weeks and placenta previa (Baumann et al. 2000). The cumulative risk could be calculated mathematically. In another study, each risk factor was given a score from 1 to 3 depending of the odds ratio and a total score was calculated (Lindqvist and Happach. 2006). The potential limitation of these analyses is that both studies are retrospective. These models may be useful in future management of high risk pregnancies if validated in prospective studies (Baumann et al. 2000, Lindqvist and Happach. 2006).

AIMS OF THE STUDY

The aims of this thesis were to determine

1. the incidence of placental abruption in a tertiary university hospital
2. the risk factors occurring before and during pregnancy ending in placental abruption
3. the clinical presentation of placental abruption
4. if placental abruption could be predicted by second-trimester maternal serum alphafetoprotein, free beta human chorionic gonadotrophin, soluble endoglin, soluble fms-like tyrosine kinase 1 and placental growth factor levels
5. if placental abruption could be predicted by first trimester maternal serum C-reactive protein and chlamydial antibody levels

SUBJECTS AND METHODS

The studies were conducted with the approval of the Ethics Committee of the Department of Obstetrics and Gynecology, Helsinki University Central Hospital. Study V was conducted in collaboration with the National Public Health Institute, Oulu.

Subjects

Study I and Study II

All patients with a diagnosis of placental abruption (ICD-10 O45.0, O45.8, O45.9) were sought among a total of 46,742 deliveries during 1997-2001 in Helsinki University Central Hospital (Women's Clinic and Maternity Hospital). The diagnosis was based on clinical symptoms and signs and ultrasound examination, and was confirmed by the presence on one or more of the following signs: postpartal retroplacental hematoma, Couvelaire uterus, or intrauterine hematoma detected at cesarean section. Women delivering after 22 weeks of gestation or having a newborn weighing more than 500g were included in the analysis. The duration of the gestation calculated from the last menstrual period was confirmed based on ultrasound screening examination performed at 11-13 weeks. The control group consisted of the next women who gave birth before and after each index case, and who had no evidence of placental abruption. Both groups included twin pregnancies. A total of 198 women with placental abruption and 396 control women were identified and included in the study (Table 5).

Study III and Study IV

All patients with a diagnosis of placental abruption were sought in Helsinki University Central Hospital (Women's Clinic and Maternity Hospital) database of 27,569 deliveries during 1997-1999. Until 1999 maternal serum screening at 15-16 gestational weeks was offered to the whole population to detect trisomy 21 and AFP-associated fetal malformations using AFP and free β -hCG as markers. Placental abruption was diagnosed and duration of the gestation was calculated as described above. Women delivering after 22 weeks of gestation or having a newborn weighing more than 500g were included. The control group consisted of the two women who gave birth before and after each index case, and who had no evidence of placental abruption. Women with multiple gestation were excluded. Among the study population none of the newborns showed evidence of chromosomal abnormalities, neural tube defects, ventral wall

defects, or congenital nephrosis. A total of 118 women with placental abruption and 236 control women were identified. Of these, 57 case women and 108 control women were from Helsinki city area and had MSAFP and MS β -hCG measured and results available (Study III) and 42 cases with subsequent placental abruption and 50 controls had serum samples collected for 21 trisomy screening and had sera available for analyses of sEng, sFlt-1 and PlGF (Study IV) (Table 5).

Study V

Cases and controls were selected as described in Studies I and II. Twin pregnancies were excluded from the both groups, and only women with normal pregnancies (i.e. deliveries ≥ 37 gestational weeks without signs of PIH, preeclampsia, chronic hypertension, IUGR, bleeding in II/III trimester, chorioamnionitis or stillbirth) were included as controls. Thus, the final study population consisted of 181 women with subsequent placental abruption and 261 control women (Table 5). Serum samples were collected at 10-11 gestational weeks.

Table 5. Number of cases and controls in each study

Study	Time period	Cases	Controls
I & II	1997-2001	198	396
III	1997-1999	57	108
IV	1997-1999	42	50
V	1997-2001	181	261

Methods

Handling of clinical data

Relevant clinical data were recorded from the hospital charts. Chronic hypertension was defined as blood pressure $\geq 140/90$ mmHg before pregnancy or before the 20th week of gestation. PIH was diagnosed if systolic blood pressure had increased by more than 30 mmHg or diastolic blood pressure by more than 15 mmHg after 20th gestational week exceeding 140/90 mmHg, in the absence of proteinuria (<0.3 g/L). Pre-eclampsia was defined as PIH with proteinuria (≥ 0.3 g/L). SGA or IUGR was defined as birth weight under the 10th percentile of the national standard (Pihkala et al. 1989). Birth before 37 completed gestational weeks was defined as preterm. First trimester bleeding was defined as bleeding before the 12th completed gestational week. Second trimester bleeding was defined as bleeding between the 12th and 28th gestational weeks, and third trimester bleeding as bleeding after the 28th week not immediately associated with placental abruption. Placental localization was recorded during the screening ultrasound examination at 18-20 weeks of gestation and those with suspicion of placenta previa were re-examined during the third trimester. Acute chorioamnionitis was defined on the basis of symptoms including maternal fever of $\geq 38^{\circ}\text{C}$, increased heart rate of the mother and the fetus, uterine tenderness, foul odor of the amniotic fluid, increased blood white cell count, and increased CRP concentration. Preterm PROM was defined as a spontaneous rupture of the membranes before 37 completed gestational weeks. Preterm PROM lasting more than 24 hours before delivery was considered prolonged. PMR referred to intrauterine fetal death or newborns surviving < 7 days. Smoking habits of the women and their partners and alcohol consumption of the women were systematically recorded at the first antenatal clinic visit. All women and their partners who smoked at least one cigarette per day were defined as smokers. Women who used more than two doses of alcohol per week were defined as alcohol users.

Occupational level or socio-economic position was defined as higher (upper level administrative, managerial or professional employees) or lower (lower level administrative or clerical employees, skilled and unskilled manual workers and unclassified workers such as unemployed, students, unknown occupation). Uterine malformations included uterine septum (partial or complete), arcuate uterus, bicornuate uterus, didelphic uterus and unicornuate uterus. Uterine malformations were diagnosed on the bases of pelvic examination, transvaginal ultrasound and operative interventions. Subfertility referred to patients who had been examined for infertility or who had used assisted reproductive technology. Assisted conception referred to patients who had

undergone ovulation induction, intrauterine insemination, standard in-vitro fertilization or intracytoplasmic sperm injection.

Assays

Established assays were employed for the determination of a given marker, and the main principles of the assays are shown in Table 6.

Alpha-fetoprotein and free beta-human chorionic gonadotrophin

Blood samples were collected from the antecubital vein. Sera were separated by centrifugation and stored at +5 °C and analyzed within 24 h. MSAFP and MS β -hCG were measured by AutoDelfia automatic immunoassay system and fluoroimmunometric hAFP/free hCGbeta Dual Kit (Perkin Elmer/Wallac, Turku). According to information from the manufacturer, the minimal detectable dose for AFP was 0.1 mU/L and for β -hCG 0.2 ng/ml. The intra- and interassay coefficients of variation at the concentrations measured were below 4.4%. All serum samples were analyzed in a single central laboratory. The patients with a risk of 1:350 or higher for Down's syndrome or an MSAFP-level > 2.5 multiples of median (MoM) were offered counseling and amniocentesis.

Soluble endoglin, soluble fms-like tyrosine kinase 1 and placental growth factor

Sera were separated by centrifugation and stored at -20 °C. Serum specimens were randomly allocated to batches and analyzed by enzyme-linked immunosorbent assays (ELISAs) for human sEng, human sFlt-1 (sVEGF R1) and human PlGF, according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, USA). All samples were run in duplicates by a technician blinded to pregnancy outcome, and the mean values of the duplicate samples were reported. According to information from the manufacturer, the minimal detectable dose for sEng was 0.007 ng/ml and for PlGF 7 pg/ml while that for sFlt-1 was 5 pg/ml. The intra- and interassay coefficients of variation at the concentrations measured were below 10%.

C-reactive protein and chlamydial antibodies

Serum samples of all pregnant women are collected during the first antenatal clinic visit (mean 10.4 gestational weeks) for routine screening, and stored at -25 °C at the National Public Health Institute serum bank. This serum bank covers over 98% of all pregnant women in Finland since 1983. Serum CRP levels were quantified using an immunofluorometric CRP kit (Innotrac Diag, Turku, Finland). The sensitivity of the assay is 0.05 mg/L and its assay range is 0.05-50 mg/L.

The intra- and interassay coefficients of variation at the concentrations measured were below 18%. Antibody analysis was done by randomly allocating the serum specimens to batches and running in duplicates blinded to pregnancy outcome. Microimmunofluorescence test (AniLabsystems, Helsinki) was used to analyze *C. pneumoniae* specific immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies. A cut-off titer >16 was defined as a positive result. *C. trachomatis* specific IgG and IgA and chlamydial heat shock protein 60 (CHSP60) specific IgG serum antibodies were analyzed by enzyme linked immunosorbent assays (Elisa) kits (Medac Diagnostika, Hamburg, Germany). Results were obtained as a mean absorbance of duplicated samples at 450 nm. Less than 10% variation was seen in doublets (optical density [OD] >0.2). Cut-off for a positive antibody level (=mean OD value of the negative control +0.350) was defined as OD>0.4. The intra- and interassay coefficients of variation at the concentrations measured were below 22% for *C. trachomatis* and CHSP60 IgG antibodies, and below 18% for IgA antibodies.

Table 6. Assay characteristics

Marker	Assay principle	Source of reagent
Alphafetoprotein	Fluoroimmunometric assay	Perkin Elmer/Wallac, Turku
β-human chorionic gonadotrophin	Fluoroimmunometric assay	Perkin Elmer/Wallac, Turku
Soluble endoglin	Enzyme-linked immunosorbent assay	R&D Systems, Inc., Minneapolis
Soluble fms-like tyrosine kinase 1	Enzyme-linked immunosorbent assay	R&D Systems, Inc., Minneapolis
Placental growth factor	Enzyme-linked immunosorbent assay	R&D Systems, Inc., Minneapolis
C-reactive protein	Fluoroimmunometric assay	Innotrac Diag, Turku
<i>Chlamydia trachomatis</i> IgG	Enzyme-linked immunosorbent assay	Medac Diagnostika, Hamburg
<i>C. trachomatis</i> IgA	Enzyme-linked immunosorbent assay	Medac Diagnostika, Hamburg
<i>C. trachomatis</i> heat-shock protein 60	Enzyme-linked immunosorbent assay	Medac Diagnostika, Hamburg
<i>C. pneumoniae</i> IgG	Microimmunofluorescence assay	AniLabsystems, Helsinki
<i>C. pneumoniae</i> IgA	Microimmunofluorescence assay	AniLabsystems, Helsinki

Statistical analyses

Categorical data were analysed by χ^2 -test, Chi-square test, or Fisher's exact probability test (Studies I-II). To compare continuous variables, Student's t-test was applied for normal distributions and Mann-Whitney U-test for other types of distributions (Studies I-II). In Study I, a multivariate logistic regression analysis was performed with placental abruption of the index pregnancy as dependent variable and demographic and historic variables as independent variables (Study I). All variables significant ($p < 0.05$) by univariate analysis were entered into the multivariate analysis. In Study II, a multivariate logistic regression analysis was performed with placental abruption as the dependent variable and selected features of the index pregnancy as independent variables. The calculations were performed with NCSS 2001 (NCSS Inc., Kaysville UT, USA).

In Study III, categorical data were analyzed by Chi-square test or Fisher's exact probability test. Continuous variables (e.g. MSAFP and MS β -hCG) with skewed distribution were analyzed either with non-parametric tests or with parametric tests after logarithmic transformation. P-values of < 0.05 were considered statistically significant. A logistic regression analysis was performed with placental abruption as the dependent variable. Variables with a p-value ≤ 0.2 in univariate analysis were used as independent variables. Binormal receiver operating characteristic (ROC) curve was constructed to assess usefulness of various cutoffs of MSAFP. The calculations were performed with NCSS 2004 (NCSS Inc., Kaysville, UT, USA).

In Study IV, categorical data were analyzed by the Chi-square test. Continuous variables with normal distribution (age, body mass index and gestational age at screening) were analyzed by the Student's t-test. The angiogenic factors were analyzed with the Mann-Whitney U-test. P-values of < 0.05 were considered statistically significant. The calculations were performed with NCSS 2004 (NCSS Inc., Kaysville, UT, USA).

In Study V, Chi-square test was used to compare categorical variables between the study groups. Continuous variables were compared by Student's t-test. Distribution of CRP levels was skewed and the comparisons were therefore done by non-parametric Mann-Whitney U-test. P-values of < 0.05 were considered statistically significant. Logistic regression analysis was used to estimate the risk of placental abruption in relation to elevated CRP levels (upper quartile) and potential confounding factors. The calculations were performed with SPSS for Windows 14.0.1 software 2006 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Detailed results are given in the original publications and only the main results are summarized here.

Prepregnancy risk factors for placental abruption (I)

The overall incidence of placental abruption was 0.42%. Placental abruption recurred in 8.8% of the cases. Univariate analysis showed that compared to controls, women with placental abruption were significantly more often older than 35 years (OR 1.7; 95% CI 1.1, 2.6), had lower occupational level (OR 1.5; 95% CI 1.0, 2.1), were more often unmarried (OR 1.4; 95% CI 1.0, 2.0), and had more often had three or more deliveries (OR 2.6; 95% CI 1.4, 4.7). Smoking before pregnancy was strongly associated (OR 2.0; 95% CI 1.3, 3.0) with placental abruption (Tables 7). Of selected gynecologic history variables uterine malformation (OR 9.4; 95% CI 2.0, 44) and history of spontaneous abortion (OR 1.6; 95% CI 1.1, 2.4) were more common among cases by univariate analysis (Table 7).

Chronic hypertension, coagulopathies, diabetes (types 1 or 2 or gestational) and subfertility occurred equally commonly in both groups. Similarly, family history of hypertension, cardiovascular diseases, diabetes, deep venous thrombosis or thromboembolism did not differ between the groups.

Table 7. Selected baseline and gynecologic history variables of the study population

Characteristic	Cases n=198 No. (%)	Controls n=396 No. (%)
Age (years)	31.3±5.8	30.5±5.4
<20	8 (4.0)	11 (2.8)
>35	50 (25.3)	66 (16.7)*
Lower occupational level	132 (66.7)	228 (57.6)*
Unmarried	84 (42.4)	135 (34.1)*
Parity		
0	84 (42.4)	187 (47.2)
1-2	89 (44.9)	188 (47.5)
≥3	25 (12.6)	21 (5.3)*
Body mass index (kg/m ²)	23.0±4.6	23.1±4.4
<19	17 (8.6)	33 (8.3)
>25	43 (21.7)	85 (21.5)
Smoker	54 (27.3)	63 (15.9)*
Uterine malformation	9 (4.5)	2 (0.5)*
Spontaneous abortion	58 (29.3)	81 (20.5)*
Induced abortion	35 (17.7)	66 (16.7)
Dilatation and curettage	71 (35.9)	120 (30.3)

*p<0.05

A total of 114 of the case women and 209 of the control women had delivered before (Table 8). Regarding the course of previous pregnancies, only history of cesarean section (when not performed because of placental abruption) (OR 1.9; 95% CI 1.2, 3.0) and history of placental abruption (OR 7.0; 95% CI 1.9, 26) were more common in the abruption group than in the control group by univariate analysis (Table 8). IUGR and preterm labor (when not related to earlier placental abruption) were equally common in both groups.

Table 8. Selected obstetric history variables of the parous women

Variable	Cases n=114 No. (%)	Controls n=209 No. (%)
Postpartum curettage	4 (3.5)	5 (2.4)
Cesarean section**	40 (35.1)	47 (22.5)*
Placenta previa	1 (0.9)	1 (0.5)
Preeclampsia or PIH	21 (18.4)	27 (12.9)
IUGR**	13 (11.4)	16 (7.7)
Intrauterine fetal death	5 (4.4)	5 (2.4)
Preterm labor <37 wk**	14 (12.3)	15 (7.2)
Placental abruption	10 (8.8)	3 (1.4)*

*p<0.05

** Cases with history of previous placental abruption were excluded.

PIH, pregnancy induced hypertension; IUGR, intrauterine growth restriction

Table 9. Multivariate analysis of the risk factors for placental abruption

Variable	Adjusted OR (95% CI)	p-value
Age >35 years	1.4 (0.9, 2.3)	0.1
Lower occupational level	1.3 (0.9, 1.9)	0.2
Unmarried	1.2 (0.8, 1.8)	0.3
Parity ≥3	1.6 (0.8, 3.1)	0.2
Smoking	1.7 (1.1, 2.7)	0.03
Uterine malformation	8.1 (1.7, 40.0)	0.003
Spontaneous abortion	1.4 (0.9, 2.1)	0.2
Previous cesarean section**	1.7 (1.1, 2.8)	0.03
Previous placental abruption	4.5 (1.1, 18.0)	0.02

** Cases with history of previous placental abruption were excluded.

Multivariate logistic regression analysis was performed by including all variables associated with placental abruption by univariate analysis (Table 9). In the adjusted analysis, smoking (OR 1.7; 95% CI 1.1, 2.7), uterine malformations (OR 8.1; 95% CI 1.7, 40), history of cesarean section (for reasons other than abruption) (OR 1.7; 95% CI 1.1, 2.8), and history of placental abruption (OR 4.5; 95% CI 1.1, 18) remained significant independent risk factors for placental abruption.

Clinical presentation and risk factors for placental abruption during the pregnancy (II)

Placental abruption was classified as total in 13 cases (7%) and partial in 185 cases (93%). Overall, 117 (59%) of the case women delivered preterm, compared to 40 (10%) of the control women (OR 12.9, 95% CI 8.3, 19.8). Of the case women, 180 (91%) delivered by cesarean section compared to 95 (24%) of the control women (OR 34.7, 95% CI 20.0, 60.1). Of the newborns with placental abruption, 51 (25%) were growth restricted compared to 16 (4%) of the control babies (OR 7.9, 95% CI 4.4, 14.3). Ten infants (4.8%) died in utero, as compared with 2 infants (0.5%) in the control group. The perinatal mortality rate was 9.2% (19 of 207), as compared with 1% (4 of 404) in the control group (OR 10.1, 95% CI 3.4, 30.1).

Univariate analyses showed that compared to the controls, the case women (OR 2.1, 95% CI 1.4, 3.3) and their partners (OR 2.3, 95% CI 1.4, 3.6) more often were smokers through the pregnancy. If both partners were smokers the risk for placental abruption was 4.8-fold (95% CI 2.2, 10.0). After antenatal clinic counseling, only 11% of the smoking women stopped smoking during pregnancy.

Case women used alcohol more often (OR 2.6, 95% CI 1.3, 5.0). Other risk factors associated with placental abruption were vaginal bleeding during the second (OR 2.4, 95% CI 1.3, 4.4) or third trimester (OR 2.5, 95% CI 1.1, 5.6), placenta praevia (OR 6.2, 95% CI 1.7, 23.3), preeclampsia (OR 2.7, 95% CI 1.3, 5.4), and chorioamnionitis (OR 3.3, 95% CI 1.1, 10.2) (Table 10 and 11).

Table 10. Selected characteristics related to the course of the index pregnancy

Variable	Cases n=198 No. (%)	Controls n=396 No. (%)
Assisted conception	13 (6.6)	24 (6.1)
Behavioral		
Smoking by		
Woman **	50 (25.3)	54 (13.6)*
Partner	40 (20.2)	40 (10.1)*
Both	22 (11.1)	10 (2.5)*
Alcohol consumption	20 (11.0)	18 (4.6)*
Pregnancy complications		
Bleeding I trimester	16 (8.1)	22 (5.6)
Bleeding II trimester	22 (11.1)	20 (5.1)*
Bleeding III trimester	13 (6.6)	11 (2.8)*
Placenta previa	9 (4.5)	3 (0.8)*
Chronic hypertension	9 (4.5)	19 (4.8)
PIH	18 (9.1)	22 (5.6)
Preeclampsia	19 (9.6)	15 (3.8)*
Maternal diabetes		
Type I or II	2 (1.0)	6 (1.5)
Gestational	18 (9.1)	43 (10.9)
Prolonged PPROM	10 (5.1)	10 (2.5)
Chorioamnionitis	8 (4.0)	5 (1.3)*
Twin pregnancy	9 (4.5)	8 (2.0)

*p<0.05

**Both groups include 11 single mothers

PIH, pregnancy induced hypertension; PPROM, preterm premature rupture of the membranes

Table 11. Multivariate analysis of the risk factors for placental abruption

Variable	Adjusted OR (95% CI)
Maternal smoking	1.8 (1.1, 2.9)
Paternal smoking	2.2 (1.3, 3.6)
Use of alcohol	2.2 (1.1, 4.4)
Bleeding in II or III trimesters	1.7 (0.9, 3.0)
Placenta previa	5.7 (1.4, 23.1)
Preeclampsia	2.7 (1.3, 5.6)
Chorioamnionitis	3.3 (1.0, 10.0)

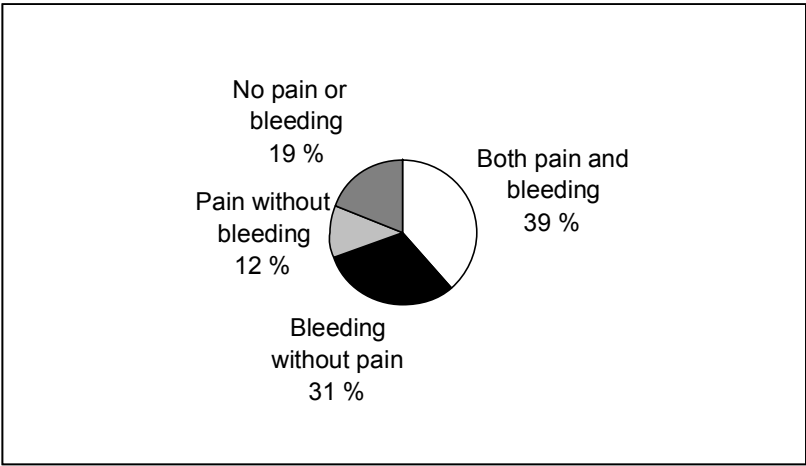
A multivariate logistic regression analysis was performed of all variables associated with placental abruption by univariate analyses. In the adjusted analyses, maternal smoking (OR 1.8; 95% CI 1.1, 2.9), paternal smoking (OR 2.2; 95% CI 1.3, 3.6), use of alcohol (OR 2.2; 95% CI 1.1, 4.4), placenta previa (OR 5.7; 95% CI 1.4, 23.1), preeclampsia (OR 2.7; 95% CI 1.3, 5.6), and chorioamnionitis (OR 3.3; 95% CI 1.0, 10.0) remained independently associated with placental abruption (Table 11).

The clinical manifestations of placental abruption are shown in Table 12 and Figure 3. Placental abruption occurred before labor in 146 (74%) cases and during labor in 52 (26%) cases. Vaginal bleeding was present in 138 (70%), abdominal pain, uterine tenderness, uterine tetanic contractions or hypertonic uterus in 100 (51%) cases, and bloody amniotic fluid in 93 (50%) cases. Fetal heart rate abnormalities (bradycardia, repetitive late decelerations, or decreased beat-to-beat variability) were present in 137 (69%) cases. Retroplacental blood clot was seen by ultrasound in 30 (15%) cases. Decreased fetal movements were reported in 22 (11%) cases. Seventy nine percent of the infants were born within 24 hours after the first symptoms of placental abruption, and 24% were born in less than one hour.

Table 12. Clinical manifestations of placental abruption

Characteristic	N=198 No. (%)
Placental abruption detected	
During labor	52 (26)
Before labor	146 (74)
Vaginal bleeding	138 (70)
Abdominal pain, uterine tenderness, uterine tetanic contractions, or hypertonic uterus	100 (51)
Bloody amniotic fluid	93 (50)
Fetal heart rate abnormalities	137 (69)
Retroplacental blood clot by ultrasound	30 (15)
Decreased fetal movements	22 (11)
Onset of symptoms before delivery	
<1 h	47 (24)
1-24 h	108 (55)
>24 h	22 (11)
Unknown	21 (11)
Extent of abruption	
Total	13 (7)
Partial	185 (93)

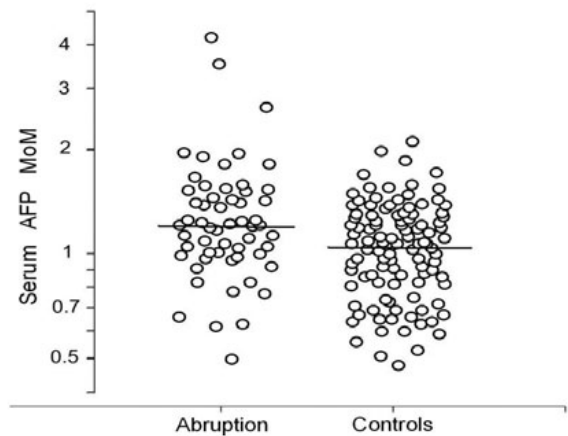
Figure 3. Pain and vaginal bleeding as symptoms of placental abruption



Alpha-fetoprotein and free beta-human chorionic gonadotrophin in prediction of placental abruption (III)

Median of the MSAFP MoM was significantly higher ($p=0.004$) in the abruption group (median 1.21, interquartile range [IQR] 1.00-1.52) compared to that in the control group (median 1.07, IQR 0.83-1.29) when adjusted for gestational age and maternal weight (Figure 4). MSAFP levels of ≥ 1.5 MoM were more common among cases (15 women, 26%) than among controls (9 women, 8%) (odds ratio [OR] 3.9; 95% confidence interval [CI] 1.6-9.7). In contrast, median of the MS β -hCG MoM did not differ between the groups (median 0.94, IQR 0.58-1.49 versus 0.89, IQR 0.61-1.37).

Figure 4. Second trimester maternal serum alpha-fetoprotein concentrations (serum AFP multiples of median [MoM] in patients with placental abruption and in controls. (y-axis: logarithmic scale). Horizontal lines indicate the median of MSAFP MoM values.



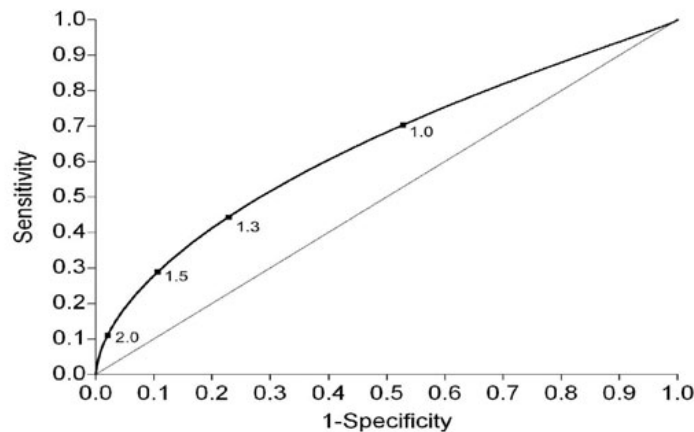
A multivariate logistic regression analysis was performed by including into the model all variables with $p\text{-value} \leq 0.2$ in the univariate analysis (Table 13). In the adjusted analysis $\text{MSAFP} \geq 1.5 \text{ MoM}$ (OR 4.5; 95% CI 1.7-11.9) remained as a significant independent risk factor for placental abruption (Table 13).

Table 13. Multivariate analysis of the risk factors for placental abruption

Variable	Adjusted OR (95% CI)	p-value
MSAFP $\geq 1.5 \text{ MoM}$	4.5 (1.7, 11.9)	0.003
Parity ≥ 3	0.9 (0.1, 5.9)	0.9
Smoker	3.2 (1.3, 7.7)	0.009
Previous placental abruption	3.2 (0.4, 24.9)	0.26
Preeclampsia	4.8 (0.8, 29.9)	0.09
Bleeding in II/III trimester	4.8 (1.4, 16.5)	0.01
Placenta previa	9.1 (0.8, 101.9)	0.07

A cutoff value of MSAFP which would best discriminate between the cases and the controls was attempted to define. However, using ROC curve analysis it was unable to identify any useful cutoff value suitable for screening. For example, the cutoff ≥ 1.5 MoM showed a sensitivity of only 29% with 10% false positive rate and cutoff ≥ 2.0 MoM showed a sensitivity of only 11% with 2% false positive rate (Figure 5).

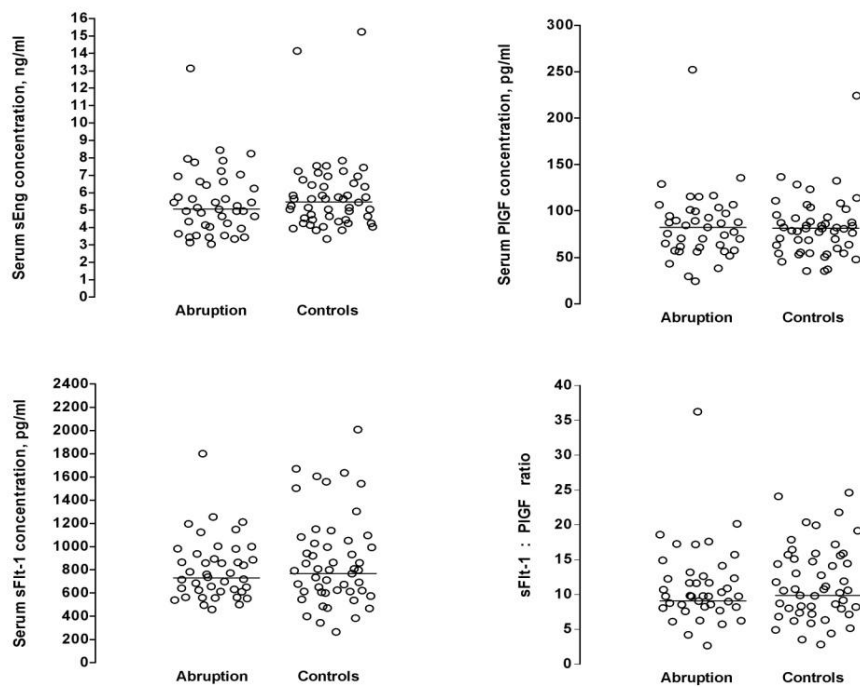
Figure 5. Receiver operating characteristic (ROC) curve showing the sensitivity and false-positive rate (1-specificity) of second trimester maternal serum alpha-fetoprotein (MSAFP) concentrations in predicting placental abruption. The numbers on the curve denote various cutoff points of MSAFP.



Angiogenic factors in prediction of placental abruption (IV)

The levels of sEng ranged from 3.0 to 13.1 ng/ml in cases and from 3.3 to 15.2 ng/ml in controls. Their medians (5.1 ng/ml vs. 5.5 ng/ml) or interquartile ranges (IQR 4.0-6.6 ng/ml vs. 4.4-6.6 ng/ml) showed no difference between the groups ($p=0.3$) (Figure 6).

Figure 6. Serum levels of soluble endoglin (sEng), soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF) and sFlt-1/PlGF ratio at gestational weeks 15 to 16 in women with placental abruption and in control women



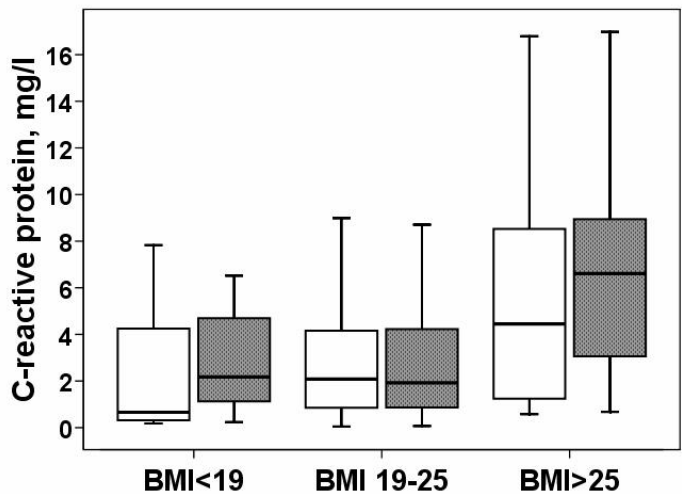
The levels of sEng were not related to the time from serum sampling to the abruption (14-26 weeks) ($r=0.06$; $p=0.7$). Parous women with abruption had lower sEng levels than parous controls [median 4.6 (IQR 3.5-5.4) vs. 5.4 (IQR 4.5-7.0), $p<0.05$]. Moreover, the levels of sEng in hypertensive women with abruption were not different from those in normotensive women. Neither depended the levels of sEng on the maternal age nor subsequent development of SGA in either group (data not shown). Within cases, smoking women had lower sEng levels than non-smoking women [median 4.3 (IQR 3.5-5.1) vs. 5.6 (IQR 4.9-7.1), $p<0.05$], and parous women had lower sEng levels than nulliparous women [median 4.6 [IQR 3.5-5.4) vs. 5.7 (IQR 4.7-7.5), $p<0.05$]. Within controls, women with smaller body mass index (≤ 22) had higher sEng levels than women with higher body mass index (>22) [median 6.0 (IQR 5.0-7.0) vs. 4.8 (IQR 4.2-5.8), $p<0.05$].

The levels of sFlt-1 [median 743.0 pg/ml (IQR 606.7-942.7 pg/ml) vs. 792.9 pg/ml (IQR 605.9-1053.6 pg/ml), $p=0.6$] or PlGF [median 82.8 pg/ml (IQR 58.6-99.8 pg/ml) vs. 80.3 (IQR 58.0-93.3), $p=0.7$] showed no difference between the study groups (Figure 1). The levels of sEng correlated negatively with the levels of PlGF ($r=-0.31$; $p=0.048$) and positively to the sFlt-1/PlGF ratios ($r=0.73$; $p<0.001$) in the abruption group. In the control group sEng was not related to PlGF but it correlated positively with sFlt-1 ($r=0.38$; $p=0.007$) and sFlt-1/PlGF ratio ($r=0.34$; $p=0.02$).

C-reactive protein and chlamydial antibodies in placental abruption (V)

There was no difference in the CRP levels between the cases and the controls (median 2.35 mg/l [IQR 1.09-5.93] vs. 2.28 mg/l [IQR 0.92-5.01], NS). As expected, the CRP levels were higher in obese women (body mass index [BMI] >25) both in the abruption and in the control group (median 6.82 mg/l [IQR 3.25-9.65] vs. 4.98 mg/l [IQR 1.25-9.77], NS) compared to lean women (BMI <19) (median 2.18 mg/l, [IQR 1.04-4.73] vs. 1.45 mg/l [IQR 0.32-5.25], NS) or women with normal BMI (median 1.95 mg/l [IQR 0.87-4.44] vs. 2.10 mg/l [IQR 0.86-4.24], NS) (Figure 7), but there was no difference between the study groups. The CRP levels were then compared in the abruption group between the smoking and the non-smoking cases, cases with or without preterm birth, preeclampsia or IUGR newborn. No differences were found in the CRP levels between these groups (data not shown). The estimated risk of placental abruption in relation to elevated CRP (upper quartile, >5.4 mg/l, OR 1.3; 95% CI 0.9-2.1) remained unchanged after adjusting for age and smoking (OR 1.3; 95% CI 0.9-2.1).

Figure 7. CRP levels in women with subsequent placental abruption (grey bars) and in women with normal pregnancy (white bars) by BMI



C. pneumoniae specific IgG and IgA antibodies and *C. trachomatis* specific IgG and IgA as well as CHSP60 specific IgG antibody prevalence rates were similar in both groups (Table 14). Similarly, CRP levels did not differ in relation to chlamydial antibodies (data not shown).

Table 14. CRP levels and antibody prevalence rates to *C. trachomatis*, CHSP60 or to *C. pneumoniae* in the cases and controls

Variable	Cases n=181 No. (%)	Controls n=261 No. (%)	p-value
CRP median (IQR)	2.35 (1.09-5.93)	2.28 (0.92-5.01)	NS
<i>C. trachomatis</i> IgG	27 (14.9)	36 (13.8)	NS
<i>C. trachomatis</i> IgA	8 (4.4)	7 (2.7)	NS
CHSP60	29 (16.0)	40 (15.4)	NS
<i>C. pneumoniae</i> IgG	98 (54.1)	146 (55.6)	NS
<i>C. pneumoniae</i> IgA	25 (13.8)	34 (13.0)	NS

DISCUSSION

Significant progress in perinatal medicine has taken place during the last few decades. This includes e.g. sophisticated ultrasound equipments and delicate methods to assess fetal oxygenation and other wellbeing, and for a good reason we can say that a fetus has become a patient for perinatologists. As a result, perinatal mortality and morbidity have dramatically decreased in the Western world (Konje and Taylor. 2001). It is unfortunate that this favorable development hardly concerns placental abruption. Although maternal and perinatal mortality rates have decreased, placental abruption still manifests itself almost similarly as in 1775 when first described by Edward Rigby (Rigby. 1775, Eskes. 1997) and the impact of this complication on other perinatal outcome is much larger than approximated from its rate. Therefore new data are needed on placental abruption and this was the purpose of the present study.

In this study the rate of placental abruption (0.42%) was twice as high as that (0.21%) reported from another university hospital during 1962-1981 in Finland (Ylä-Outinen et al. 1987). This suggests that the rate of placental abruption may have increased. An increase has also been reported from other countries (Saftlas et al. 1991, Rasmussen et al. 1996). However, our rate of placental abruption is lower than reported from elsewhere (0.5-0.6%) (Ananth et al. 1999a, Ananth et al. 1999b, Baumann et al. 2000, Ananth and Wilcox. 2001, Kyrklund-Blomberg et al. 2001, Toivonen et al. 2002) but higher than in a recent large Norwegian study (0.38 %) (Nilsen et al. 2008). The prevalence of placental abruption seems to be lower in the Nordic countries than in the U.S. This could reflect differences in diagnostic criteria, different study populations, or different recording of placental abruption. For instance, in many studies the lowest gestational age considered has not been defined or mentioned (Kåregård and Gennser. 1986, Saftlas et al. 1991, Rasmussen et al. 1996, Ananth et al. 1999b, Kyrklund-Blomberg et al. 2001). Also, in our study small abruptions may not have been recorded in hospital charts or may have been recorded under different diagnoses. However, it is unlikely that this bias may have changed the key findings of our study.

Risk factors associated with placental abruption

Although univariate analysis identified many risk factors, only smoking, uterine malformation, previous cesarean section and history of placental abruption were significantly associated with the increased risk of placental abruption in a subsequent pregnancy. During the index pregnancy independent risk factors for placental abruption were maternal and paternal smoking, use of alcohol, placenta previa, preeclampsia, and chorioamnionitis.

Smoking was the strongest risk factor for placental abruption. A meta-analysis showed that smoking increases the risk of placental abruption by 90% and 15-25% of episodes could be prevented if women stopped smoking during pregnancy (Ananth et al. 1999a). In our series maternal smoking doubled the risk of placental abruption. An interesting new finding was that also paternal smoking was an independent risk factor irrespective of whether the women smoked. This can be explained by passive exposure of the pregnant woman to partner's smoking, or that women whose partners smoked also smoked despite claiming of being nonsmokers. A Finnish study based on cotinine assessment suggests that about one out of four smoking parturients deny smoking (Bardy et al. 1993). Smoking by both partners, which increased the risk nearly 5-fold, may reflect a more liberal attitude towards smoking in the family. The mechanisms explaining the association between smoking and placental abruption may include decidual necrosis at the margins of the placenta, placental microinfarcts, atheromatous or fibrinoid changes, or the development of hypovascular and atrophic placental villi (Naeye. 1980, Andres and Day. 2000). Increased capillary fragility may result in arterial rupture leading to placental abruption (Kaminsky et al. 2007). Although women who stop smoking early in pregnancy have similar risk of abruption than nonsmoking women (Naeye. 1980, Ananth and Cnattingius. 2007), smoking cessation during pregnancy is problematic. In one study, smoking discontinuation rate was 18% (Lumley. 1987). In our study only 7% of smoking women with subsequent abruption and 14% of smoking control women were able to stop smoking during pregnancy. Most of the studies are based on self-reported smoking behavior (Ananth et al. 1999a). Social stigma associated with smoking may lead to underreporting. Studies using biochemical markers such as cotinine assessment, the primary metabolite of nicotine, suggest that women consistently underreport smoking behavior (Bardy et al. 1993, Moore et al. 2002). Clearly, stopping smoking is the best intervention to reduce perinatal mortality and morbidity (Pfarrer et al. 1999).

Uterine anomalies, caused by abnormal fusion of the Müllerian ducts (Heinonen et al. 2000), are found in 0.1-2% of all women (Acien. 1997). We found an association between placental abruption and congenital malformations of the uterus. Although mentioned in some old textbooks (Green. 1989), none of the most recent studies have confirmed this association. In our study women with uterine malformation had 8-fold risk for placental abruption. Uterine malformation may lead to poor decidualization and placentation. Also the contractility of malformed uterus may be disturbed or uncoordinated increasing the risk for preterm separation of the placenta (Dabirashrafi et al. 1995) . However, it is possible that in our study malformed

uterus may have been more readily diagnosed among cases who underwent cesarean deliveries or had increased rate of spontaneous abortions, resulting in reporting bias.

Past cesarean section increases the risk of placental abruption by approximately 40% and short interpregnancy interval after cesarean section further increases this risk (Hemminki and Meriläinen. 1996, Rasmussen et al. 1999, Getahun et al. 2006, Yang et al. 2007). In our study, the risk of placental abruption associated with history of cesarean section was almost twofold. It is likely that uterine low segment scar may impair placental attachment and perfusion in anteriorly located placentas, and therefore an increase in the risk of abruption is seen (Rasmussen et al. 1999, Lydon-Rochelle et al. 2001). Also, ligation of uterine vessels at the time of cesarean section may further damage endometrial and myometrial lining. Not surprisingly, history of cesarean section also increases the risk of stillbirth and placenta previa in subsequent pregnancies (Smith et al. 2003, Getahun et al. 2006, Yang et al. 2007).

The recurrence rate of placental abruption was 8.8 % in our study, which is in line with previous studies (Kåregård and Gennser. 1986, Rasmussen et al. 1997, Konje and Taylor. 2001). It is not known why placental abruption recurs in some women. Recurrence cannot be only explained by the common risk factors (Ananth and Cnattingius. 2007). Placental abruption may simply cause a permanent damage to the endometrium which then increases the risk of inappropriate placentation in subsequent pregnancy (Ananth et al. 1996). Genetic predisposition or immunological factors may also play a role. Thus, a history of placental abruption should alert of an increased risk of subsequent placental abruption. Testing placental function and assessment of fetal growth six weeks before the time of previous placental abruption has been advocated in order to lower the risk for recurrent placental abruption (Rasmussen et al. 2001).

Alcohol use doubled the risk for placental abruption in our study, and this is in line with some (Kaminski et al. 1976, Halmesmäki. 1988, Burd et al. 2007), but not all studies (Kramer et al. 1997). Alcohol easily crosses placenta and accumulates in the fetus and amniotic fluid (Burd et al. 2007). Alcohol use during pregnancy is a known risk factor for fetal neurodevelopmental abnormalities and fetal malformations (Kaminski et al. 1976, Sokol et al. 2003). Alcohol exposure also causes vasoconstriction in the placenta and umbilical cord and disturbs the fetomaternal hormonal balance (Gabriel et al. 1998, Burd et al. 2007) which may contribute to the risk of abruption. Villus infarction and intervillous thrombosis are more common in placentas of alcohol users (Burd et al. 2007). In alcohol-exposed pregnancies both placental and fetal growth is impaired. Alcohol usage also causes intrapartum asphyxia and may lead to stillbirth (Burd et al. 2007).

Bleeding during second or third trimester and placenta previa were also risk factors for placental abruption. This is in accordance with previous studies (Kramer et al. 1997, Baumann et al. 2000, Kyrklund-Blomberg et al. 2001). The presence of a retroplacental hematoma in the first trimester is known to increase the risk for subsequent placental abruption (Sipilä et al. 1992, Nagy et al. 2003). Vaginal bleeding after 28 weeks also predicted placental abruption, as has been shown before (Baron and Hill. 1998). However, in our study bleeding in the second trimester or the third trimester was not an independent risk factor in the adjusted analyses which can be explained by the strong impact of placenta previa on placental abruption. According to the literature 10% of women with placenta previa have a coexisting abruption (Konje and Taylor. 2001), although in some studies these cases are not considered abruptions (Oyelese and Ananth. 2006).

Preeclampsia and chorioamnionitis were strong risk factors for placental abruption. Preeclampsia was associated with a 2.7-fold risk of placental abruption, while chronic hypertension or PIH was not. This is in line with previous studies (Ananth et al. 1997, Kramer et al. 1997). Preterm PROM has been associated with the risk of placental abruption (Ananth et al. 1996, Toivonen et al. 2002), which may be a consequence of ascending intrauterine infection. Another study showed that prolonged preterm PROM increased the risk of abruption even more (Ananth et al. 2004). However, this was not seen in our study. This may be due to the liberal use of prophylactic antibiotics which may prevent chorioamnionitis or to our active policy of inducing labor within 12-24 hours after preterm PROM if the duration of gestation exceeds 34 weeks. Nevertheless, chorioamnionitis increased the risk for placental abruption more than 3-fold.

Assisted conception, polyhydramnion, oligohydramnion, maternal diabetes, uterine trauma, external cephalic version, short umbilical cord, and coagulation defects have all been associated with placental abruption. None of these risk factors was associated with placental abruption in our study which may be due to small numbers. Also, our cases were not systematically tested for thrombophilic disorders. In some (Ananth et al. 2003, Campbell and Templeton. 2004) but not all studies (Kramer et al. 1997) multiple pregnancy has been a risk factor for placental abruption. This was not found in our study. Many of these additional risk factors were infrequent in our study, and potential role of these risk factors can only be addressed in large epidemiological studies.

Clinical presentation

The clinical manifestations of placental abruption can be highly variable. Vaginal bleeding was the most common symptom (70%) of placental abruption, and pain was present in half of the cases. This is in line with previous reports (Konje and Taylor. 2001). However, it is remarkable that nearly one out of five cases had neither pain nor bleeding. Yet, of these women nearly all presented with CTG changes. Only two women had retroplacental hematoma as the only sign, and both newborns were growth restricted. According to the literature up to 50% of abruptions take place during labor (Baron and Hill. 1998), but this was the case in only 27% of our patients. The liberal use of ultrasound examination and cesarean section at an early phase in patients suspected of having abruption may have contributed to this difference. More than 90% of our cases with abruption were delivered by cesarean section. This rate is much higher than in most previous studies (Kåregård and Gennser. 1986, Toivonen et al. 2002). Clear retroplacental blood clot before delivery was detected by ultrasound in only 15% of our cases. Therefore, the absence of ultrasound findings should not preclude the diagnosis. Ultrasound is not a sensitive method of diagnosing placental abruption but it is useful diagnosing coincident placenta previa (Konje and Taylor. 2001). Prematurity and intrauterine growth restriction are major problems associated with placental abruption (Ananth et al. 1999b); 59% of the case women had a preterm birth and 25% of the newborns were growth restricted. Also, perinatal mortality was increased 9-fold.

Biochemical markers

Several biochemical markers have been studied in order to predict placental abruption but none have emerged clinically useful (Nolan et al. 1993, Bartha et al. 1997, Chandra et al. 2003, Florio et al. 2003, Dugoff et al. 2005, Signore et al. 2006). Preeclampsia and placental abruption share similar placental histopathology and also insufficient uteroplacental circulation (Steinborn et al. 2004, Oyelese and Ananth. 2006). Elevated second-trimester AFP levels or β -hCG levels, and elevated sEng or sFlt-1 and decreased PlGF levels have been demonstrated in preeclampsia (Liu et al. 1999, van Rijn et al. 1999, Chandra et al. 2003, Dugoff et al. 2005) but not much is known of these markers in placental abruption. Generalized inflammatory response and endothelial cell dysfunction have also been demonstrated in preeclampsia (Bowen et al. 2001, Redman and Sargent. 2005, Ness and Sibai. 2006). In one study, CRP levels were increased in women with subsequent preeclampsia (Qiu et al. 2004a). *C. pneumoniae* has been linked to preeclampsia (Heine et al. 2003, Goulis et al. 2005). *C. trachomatis* has been linked to several adverse pregnancy outcomes (McGregor and French. 1991, Claman et al. 1995, Gencay et al. 2000, Karinen et al. 2005). Although there is some evidence that chronic inflammation is implicated in

placental abruption (Ananth et al 2006) none of these markers have been studied in placental abruption.

Elevated second-trimester MSAFP-levels or MS β -hCG-levels have been linked to many pregnancy complications but placental abruption has only represented one of many (Katz et al. 1990, Purdie et al. 1983, Liu et al. 1999, van Rijn et al. 1999, Chandra et al. 2003). After exclusion of pregnancies with fetal chromosomal or structural abnormalities, the MSAFP or the MS β -hCG cutoff level most often used for adverse perinatal outcome has been ≥ 2 or 2.5 MoM (van Rijn et al. 1999, Chandra et al. 2003, Dugoff et al. 2005). In our study the goal was to find out whether MSAFP or MS β -hCG levels could specifically predict placental abruption.

Fetal liver produces AFP. Due to renal output, AFP accumulates in amniotic fluid especially during the second trimester. From amniotic fluid, AFP enters maternal circulation transplacentally (70%) and, to some extent, transamniotically (Kraus et al. 2004). Placental abruption may often be a consequence of spiral artery rupture, causing retroplacental hematoma and then placental detachment (Haddow and Palomäki. 1999). Disruption in the feto-maternal interface in early second trimester might permit transfer of AFP into maternal circulation (Chandra et al. 2003). This blood, rich in AFP, could be responsible for elevation in MSAFP in our patients with subsequent placental abruption since the median of MSAFP was higher in women who developed placental abruption. After adjusting for potential confounding factors, MSAFP ≥ 1.5 MoM remained an independent predictor for placental abruption. However, the clinical usefulness of this test was limited due to low sensitivity and high false-positive rate. β -hCG levels did not predict placental abruption in our study.

Although the etiology of placental abruption is unknown the trophoblastic invasion in the spiral arteries and consequent early vascularization may be defective (Dommissse and Tiltman. 1992, Kraus et al. 2004). Therefore, it is possible that an imbalance between placental proangiogenic and antiangiogenic factors locally in the placenta or in maternal blood precedes placental abruption. In one study the serum levels of PlGF were decreased and the ratios of sFlt-1/PlGF were increased in nulliparous women several weeks before placental abruption, but this was the case only in women who also developed preeclampsia (Signore et al. 2006). Our data based on serum samples collected at the time of critical remodelling of the spiral arteries failed to identify women with subsequent placental abruption which is in agreement with the previous study (Signore et al. 2006). These results strongly imply that circulating proangiogenic PlGF and antiangiogenic sFlt-1 in early or midgestation do not predict placental abruption.

Endoglin, a cell membrane coreceptor for different growth factors, is produced in endothelium and syncytiotrophoblasts and acts as an important antiangiogenic factor (Venkatesha et al. 2006).

In the genetically manipulated mice the overproduction of this factor leads to insufficient general and placental angiogenesis (Li et al. 1999). The soluble form of endoglin leaking into maternal blood was markedly increased in early second trimester in women who later become preeclamptic (Levine et al. 2006). We found that the levels of sEng were normal 14-26 weeks before placental abruption. Thus, in this regard sEng behaved like sFlt-1, PlGF, or their ratio. We could not demonstrate any elevation in sEng even in women who later became hypertensive, with or without placental abruption. This can well be due to the small number of hypertensive women included in our study. Another study showed significantly elevated levels of sEng already at 17-20 gestational weeks in women with subsequent early preeclampsia (Levine et al. 2006).

Maternal smoking has been associated with reduced levels of sEng and sFlt-1 and increased levels of PlGF (Levine et al. 2006). We found that sEng levels were significantly lower in smoking women with abruption. This effect might be part of adaptive angiogenesis which means increased placental weight and impaired placental function among smoking women (Pfarrer et al. 1999).

Immunological defects may also play a role in the etiology of placental abruption (Matthiesen et al. 1995, Steinborn et al. 2003b) leading to excessive maternal inflammatory response with increased release of cytokines which results in a chain of events including shallow trophoblast invasion, defective spiral artery remodelling, placental infarctions and thrombosis (Matthiesen et al. 2005). Excessive activation of the immune system may suggest past exposure to major antigens (Steinborn et al. 2004). Chlamydiae are common pathogens and immune system modulators (Paavonen and Eggert-Kruse. 1999, Hammerschlag. 2007, Meyers et al. 2007). CRP is a sensitive marker of inflammation, and Chlamydiae can induce such an inflammation. Thus, evaluating these biomarkers in the prediction of placental abruption was meaningful.

CRP is a protein synthesized in hepatocytes. Elevated CRP values reflect the amount of circulating inflammatory cytokines and inflammation in general (Loukovaara et al. 2003, Qiu et al. 2004a). Highly sensitive CRP predicts subsequent coronary heart disease events (Danesh et al. 2004). The risk of premature cardiovascular disease is increased after maternal placental disease syndrome, i.e. hypertensive disorders, placental abruption or infarction (Ray et al. 2005). CRP concentrations correlate with BMI (Qiu et al. 2004a). We could also demonstrate the effect of BMI on CRP levels but as such CRP alone did not predict placental abruption. This implies that increased CRP levels in early pregnancy do not contribute to the pathogenesis of placental abruption. However, we cannot exclude the possibility that CRP levels increase later in gestation.

Many adults have been exposed to *C. pneumoniae* (Goulis et al. 2005). *C. pneumoniae* antibodies have been increased in women with preeclampsia in some (Heine et al. 2003, Goulis et al. 2005), but not all studies (Teran et al. 2003, Raynor et al. 2004). In preeclampsia as well as in placental abruption a common histological finding in spiral arteries is atherosclerosis, a lesion involving same lipid-laden foam cells also observed in atherosclerosis (Goulis et al. 2005, Ananth et al. 2006b). *C. pneumoniae* infection has been linked to atherosclerosis and coronary artery disease (Leinonen and Saikku. 2002). This organism has also been detected in atherosclerotic artery tissue (Leinonen and Saikku. 2002). It has been proposed that *C. pneumoniae* causes chronic inflammation, which can result in clinical disease syndromes developing years after the primary infection (Goulis et al. 2005). However, we could not demonstrate any association between *C. pneumoniae* antibodies and placental abruption.

C. trachomatis is the most common cause of bacterial sexually transmitted infections. More than 10% of women of reproductive age report a history of *C. trachomatis* infection (Gencay et al. 2000). *C. trachomatis* infection has been linked to an increased incidence of pregnancy loss, low birth weight, prematurity, preterm labor and premature rupture of the membranes (McGregor and French. 1991, Claman et al. 1995, Gencay et al. 2000, Blas et al. 2007). *C. trachomatis* IgG seropositivity indicates past, persistent or latent *C. trachomatis* infection and has also been detected more often in the sera of mothers with stillbirth (Gencay et al. 2000). One recent study suggested that chronic *C. trachomatis* infection may lead to systemic low-grade inflammation and elevated CRP levels contributing to the pathogenesis of preterm delivery (Karinen et al. 2005). *C. trachomatis* is a small intracellular, gram-negative bacterial organism. Such intracellular organisms can escape humorally mediated host defences, which may account for the typically low antibody levels in uncomplicated infection and the prolonged persistence of untreated infection (McGregor and French. 1991). Heat shock proteins (HSPs) protect cells against different forms of stress, such as hypoxia, ischemia and hyperoxia (Di Felice et al. 2005). Chlamydial heat shock proteins have been linked to the development of immunopathological damage after *C. trachomatis* infection (Karinen et al. 2005, Meyers et al. 2007). Serum antibodies to chlamydial HSPs have been associated with poor reproductive outcome and also to the development of cervical or ovarian cancer (Claman et al. 1995, Koskela et al. 2000, Anttila et al. 2001, Paavonen et al. 2003, Di Felice et al. 2005, Karinen et al. 2005). However, we failed to demonstrate any association between *Chlamydiae* and placental abruption. An excessive activation of the immune system seen in placental abruption may suggest past exposure to major microbial antigens (Steinborn et al. 2004) other than *Chlamydiae*.

Multiple risk factors for placental abruption exist, but the numbers of cases in our study were too small to test all risk factors. Also, we were unable to substantiate the association between placental abruption and coagulation abnormalities since our cases were not systematically tested for thrombophilic disorders. The limitations concerning biochemical markers were that test results from all women were not available since the blood samples had been drawn outside our hospital district. Also, for analysing angiogenic factors only early second trimester serum samples were available and only first trimester serum samples for the analysis of CRP analyses. Thus we cannot exclude the possibility that these markers might have predicted placental abruption later in pregnancy.

The strengths of our study include systematic data collection and the university hospital setting. Our university hospital is a tertiary referral hospital that serves one fifth of the Finnish female population and our department had more than 9,000 deliveries per year during the study period. Therefore, our data are likely to represent rather well the situation in the whole country. We used systematic criteria for case definition in order to avoid selection bias. The relatively large number of cases made it possible to perform multivariate analyses of the risk factors. Also, our case series was collected at the time when screening and diagnostic ultrasound examinations were already extensively used in obstetric practice. This allowed us to evaluate whether the liberal use of ultrasound had any obvious impact on the diagnosis or management of placental abruption although our study was not a randomized trial. The strength concerning biochemical marker studies was that the study was population based. The serum samples were obtained prior to the study which excludes selection bias.

In conclusion, although univariate analysis identified many prepregnancy risk factors for placental abruption, only smoking, uterine malformation, previous caesarean section and history of placental abruption remained significant by multivariate analysis. During the index pregnancy maternal alcohol consumption and smoking and smoking by the partner turned out to be the major independent risk factors for placental abruption. Smoking by both partners multiplied the risk. Other independent risk factors were coexisting placenta previa, preeclampsia and chorioamnionitis. Nearly one fifth of women with placental abruption did not have the classical symptoms of bleeding and pain. The liberal use of ultrasound examination contributed little to the management of women with placental abruption. Prematurity and intrauterine growth restriction were the major problems associated with placental abruption; 59% of the case women had a preterm birth and 25% of the newborns were growth restricted. Also, perinatal mortality was increased 9-fold. Although second-trimester MSAFP levels were higher in women with subsequent placental abruption, clinical usefulness of this test was limited due to low sensitivity

and high false positive rate. Similarly, angiogenic factors in early second trimester, or CRP levels, or chlamydial antibodies in the first trimester failed to predict placental abruption.

CONCLUSIONS

The following conclusions can be drawn:

1. Placental abruption occurred in 198 of 46 742 parturients (0.42%) in our University Central Hospital in 1997-2001. The recurrence rate of placental abruption was 8.8%.
2. Smoking (OR 1.7), uterine malformation (OR 8.1), previous cesarean section (OR 1.7) and history of placental abruption (OR 4.5) were the prepregnancy risk factors associated with placental abruption.
3. The risk factors for placental abruption during the index pregnancy were maternal (OR 1.8) and paternal (OR 2.2) smoking, alcohol use (OR 2.2), placenta previa (OR 5.4), preeclampsia (OR 2.7) and chorioamnionitis (OR 3.3). Smoking by both parents multiplied the risk (OR 4.8).
4. Vaginal bleeding, abdominal pain, bloody amniotic fluid and fetal heart rate abnormalities were the most common clinical signs of placental abruption. Traditional symptoms, bleeding and pain, were absent in 19% of the cases. Retroplacental hematoma was seen by ultrasound in 15% of the cases. Of the newborns 25% were small for gestational age, 59% were born preterm and 91% were delivered by cesarean section. The perinatal mortality rate was 9.2%.
5. An elevated maternal serum AFP 1.5 MoM was an independent risk factor for placental abruption. The clinical usefulness of this test was limited due to low sensitivity (29%) and high false positive rate (10%). Maternal serum free beta human chorionic gonadotrophin did not predict placental abruption.
6. Soluble endoglin, soluble fms-like tyrosine kinase 1 and placental growth factor did not predict placental abruption when tested 14 to 26 weeks before abruption.
7. Chlamydial antibodies and C-reactive protein did not predict placental abruption when tested in the first trimester.

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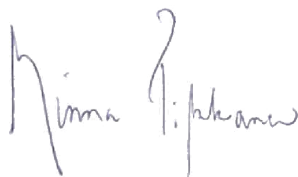
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Helsinki, May 2008

A handwritten signature in blue ink, reading "Minna Tikkanen". The signature is written in a cursive, flowing style.

Minna Tikkanen

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